SOME CYTOLOGICAL AND PHYSIOLOGICAL STUDIES OF MOSAIC DISEASES AND LEAF VARIEGATIONS[1]

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INTRODUCTION

During recent years, there has been a rising interest in the physiological, anatomical, and cytological study of the mosaic diseases of plants, as a result of the difficulty connected with a determination of the causal agency. Particularly has there been a cytological search of the tissues in an attempt to find either in the living or in the fixed and stained cell an organism which might prove to be associated with the disease. Notable among these studies have been those on tobacco by Iwanowski ('03), Hunger ('05), Delacroix ('06), Dickson ('22), Palm ('22), Goldstein ('24), Rawlins and Johnson ('24, '25), and Eckerson ('26); on potato by Smith ('24); on corn by Kunkel ('21); on the yellow stripe disease of sugar cane by Matz ('19); on the Fiji disease of sugar cane by Lyon ('10), Reinking ('21), and Kunkel ('24); on the mosaic and rosette of wheat by McKinney, Eckerson, and Webb ('23); and on *Hippeastrum equestre* Herb. by Kunkel ('22, '24), and McKinney, Eckerson and Webb ('24); and on *Brassica pekinensis* Skeels by Kunkel ('24).

In the course of these investigations various bodies and cell inclusions of different types have been described. Among other structures found in the cell have been the irregular, amoeboid-like, vacuolate or reticulate bodies which are in many ways comparable to the Negri bodies accompanying rabies, the Guarnieri bodies in small-pox, and the supposed Rickettsia micro-organisms of exanthematic typhus. These amoeboid bodies, as well as the other cell inclusions referred to, have been

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shown to be usually connected with the chlorotic areas in the mosaic-diseased plants in which they occur, and many theories regarding their probable origin and their connection with the disease have been propounded. Hence, the purpose of this investigation was to study both fixed and living cells of numerous types of mosaics in order to determine of how general occurrence the bodies are in the cells of plants affected with mosaic diseases. It was hoped that, through a study of the living cells, some index as to the physiological nature of these bodies might be obtained.

Chlorosis, the pathological condition of which the mosaic disease is a type, has been defined by Clinton ('15) as "that unusual condition in a plant in which the chlorophyll loses its bright green color and becomes yellowish green or even white." The various types of chlorosis have been classified by the same author as follows:

I. Infectious chlorosis.
  A. Communicable through the juice.
  B. Communicable through the tissues.
    a. By buds.
    b. By grafts.
II. Non-infectious chlorosis.
  A. Non-perpetuating.
    a. Affecting plants generally.
    b. Affecting isolated leaves or branches.
  B. Perpetuating.
    a. Through seed.
    b. Through cuttings.
    c. Through buds or grafts.

The mosaic diseases fall naturally in the class of infectious chloroses communicable through the juice. It was, therefore, of interest to make a cytological study of some of the other types of chloroses with the idea of determining how general an associate of chlorosis these various inclusions, and particularly the irregular vacuolate bodies, are. That is, the question arose as to whether these bodies were associated with the loss of chlorophyll in plant cells, or whether they accompanied only the infectious chloroses.

In the majority of the more common horticultural variegated varieties of plants, such as the crotens, oleander, Pittosporum, etc., chlorosis is non-infectious and is not communicable through

either the plant juices or grafting. It is perpetuated in some cases through seeds; in some, through cuttings; and in still others through buds or grafts. However, Baur ('04, '06, '07, '08) showed that there were some variegations, particularly those in the *Malvaceae*, such as *Abutilon Thompsoni*, which were infectious in nature, since in grafting the variegated variety with the green the variegation could be transmitted either from stock to scion or vice versa. There has always been some feeling that Baur's infectious chloroses are not entirely unrelated to the mosaic diseases which are simply infectious chloroses transmitted through the diseased juice. Perhaps, when more is known of Baur's infectious chloroses, it will be found that there is some means other than grafting by which they also may be transmitted.

Histological studies of variegated leaves have been made with the idea of classifying them through their anatomical structure. The outstanding work along this line is that of Funaoka ('24), in which 18 different variegations were studied and classified on the basis of characters of their microscopic anatomy. None of these studies, however, gave any indication of the presence in the cells of bodies and inclusions comparable to those found in certain of the mosaic diseases. Hence, a cytological study of representatives of the various types of variegations was planned in order to determine whether there are any inclusions in the chlorotic areas comparable to those in the chlorotic areas of the mosaic diseases studied, and if so, whether they are in any way similar. It was hoped that such a study would give some indication of a possible relation between the infectious and non-infectious variegations and the mosaic diseases which are due to, and transmitted by, the filterable virus in the diseased juice.

Discussion of Literature

A mosaic disease was first carefully studied by Mayer ('86) in tobacco, but although he found that it could be transmitted by inoculation of the mosaic juice into healthy plants and that it was inactivated by heating to 80° C., he made no cytological study of the tissue. He did examine the sap but found only starch grains and calcium oxalate crystals. Koning ('99) drew

the cross-sections of a few leaves but failed to observe any pathological inclusions. It was Iwanowski ('03) who first made a detailed cytological study of the leaves of infected plants. He studied living cells as well as cells of tissues which had been fixed in Flemming's solution, in osmic acid, and in boiling absolute alcohol, and of these he found the latter the most successful. In the living cells he observed a greater abundance of oxalate crystals in the chlorotic than in the green area; clear plate-like crystals which he described as being similar to waxy material, but less refractive; cells with granular bacteria-like inclusions which, however, never form uninterrupted complexes; and finally, plasma-like accumulations reminding one of parasitic amoebae. In the material fixed in boiling absolute alcohol and stained with methylene blue and eosin he found structures which he thought were zooglea of bacteria with approximately the same form as those of the plate-like crystals in the living cells. Also, he observed, as in the living cells, that the plasma accumulations took the eosin more deeply than did the cytoplasm, and these were most frequently found in the neighborhood of the nucleus or the crystals.

To these observations he gave the following interpretations: the plate-like crystals in the living material gave rise to the striate zooglea in fixed material, the latter being composed of organisms which were the causal agency of the mosaic and were small enough to pass through a filter; the amoeboid bodies, he believed, were the result of the reaction of the cell to the irritation of the causal agency.

Hunger ('05), in checking Iwanowski's work, showed that the so-called mosaic disease bacteria of Iwanowski, as well as the zooglea, disappeared when the cell was treated with phenol chlor-hydrate, while the other cell-structures remained the same. That is, he obtained a solution of the plate-like crystals with phenol chlor-hydrate.

Delacroix ('06), in following the method of Iwanowski of fixing the tissues in boiling absolute alcohol, was able to observe the amorphous bodies and the zooglea. However, when fixed in cold absolute alcohol he obtained no such structures. From these observations he concluded that they were deposits of

substances which were dissolved in the cell sap and precipitated by the boiling alcohol. He apparently did not account for the fact that they also appeared in the living cells. In view of this latter fact his results could no doubt be interpreted as having been brought about by the solution of the bodies in cold but not in hot absolute alcohol.

Lyon ('10), in studying the Fiji disease of sugar cane, found one or more plasmic bodies in every cell of the abnormal tissue. They were generally rounded in contour although they might be variously lobed or distorted at times, and their constituent substance was much denser optically than that of the cytoplasm of the cane, making them readily visible even though all the contents of the cells were clear and colorless. They were devoid of cell walls or other precipitated secretions. In interpreting these observations, he concluded that "In these foreign bodies we recognize an interesting and dangerous parasite quite new to the science of sugar cane pathology. It belongs to a very small group of lowly organisms whose position in the realm of living things is a subject of dispute among naturalists." Apparently he considered that they were plasmodia of a myxomycete, similar to *Plasmodiophora Brassicae*, which break up to form spores, the latter being freed with the disintegration of the tissue. Infection, then, according to him takes place by the entrance of the swarm spores through the roots and up through the vessels to the leaves.

Matz ('19), in his studies on the yellow stripe disease of sugar cane, observed granular, plasmodium-like substances in the yellow-striped cane leaf and stem tissues, and found the mass made up of small, hyaline bodies, the entire mass being in the form of a compact plasma with the bodies less than 1 μ in length. They seemed, however, less clearly defined than masses of bacteria. Later, in 1922, he considered them as co-generic with *Strongyloplasma Iwanowski* Palm, described by Palm in the chlorotic areas of the tobacco leaves infected with mosaic.

The term *Strongyloplasma Iwanowskii* was applied by Palm ('22) to the very small granules (at the most .5 μ in length) which he observed in the chlorotic areas of tobacco leaves infected with the mosaic disease. He found them staining black with haematoxylin, and frequently forming irregular agglomera-

tions which sometimes filled the entire cell lumen. These very small bodies he believed agreed in every respect with the *Strongyloplasma* of von Prowazek and Lipschütz. He also observed the fairly large corpuscles usually in contact with, or in close proximity to, the nucleus, staining gray with haematoxylin and very light red with eosin. In the living cells the latter were present and were denser and more opaque than the surrounding cell plasm. Although they were displaced at times by the streaming of the protoplasm, he saw no evidence of any automotive movement. In the interpretation of his results he agreed with Iwanowski ('03) that these latter bodies were the product of the reaction of the virus carrier on the cell plasm, and believed that they were homologous with the Guarnieri bodies. The very small granules were, according to him, the causal agency, and he therefore concluded that in the mosaic disease of tobacco we are dealing with a disease which belongs etiologically to the chlamydozoonoses.

The presence of the plasmodial bodies in the Fiji disease of sugar cane as described by Lyon ('20) was verified by Reinking ('21), who reported finding the bodies as light-colored in the younger galls and brown and granular in the older ones. He found them in the young shoots arising from the base of diseased plants, in the rotted roots, and in the base of the stem. He concluded that, although the presence of the bodies throughout the plant has not yet been demonstrated, their presence would indicate that "the fungus is responsible for the disease."

Kunkel ('24) studied the bodies in connection with the Fiji disease in great detail and found particularly in the young galls that they were frequently small and composed of a deeply staining granular mass with occasional vacuoles. In many cases, they possessed short blunt appendages which were more hyaline than the main part of the body and resembled pseudopodia of amoebae. In maturing and old galls, the bodies became larger and less dense, frequently containing large, deep-staining granules in a vacuole surrounded by a definite membrane. He frequently found two bodies, one in either end of the cell, in the case of dividing cells, thus indicating that these plasmodial bodies do possess the power of division and of growth. These facts,

together with the distribution of the bodies in the gall tissues, their apparent manner of spreading, and constant association with the earlier stages of gall formation, indicate, he believed, that they belonged to a parasitic organism. However, he still considered it an open question as to whether they were inert cell inclusions resulting from some physiological disturbance, or represented a new type of plant parasite.

Kunkel ('21) has also studied the mosaic disease of corn, and compared the bodies found there with those in the yellow stripe disease and the Fiji disease of sugar cane. Studies were made both of living and fixed material, and in all chlorotic areas irregular amoeboid bodies were found in association with the host cell nucleus and frequently appeared to be attached to it. They showed a structure similar to that of protoplasm but somewhat more dense and opaque. In the living cells, he never found them showing any automotive movements or change of form. Frequently they appeared naked, but at times a thin limiting membrane could be observed. Vacuoles were present in some and in others they were absent. Also, there were in some of the bodies numerous dark-staining granules which tended to be angular rather than spherical and never showed any semblance to nuclei. Those cells possessing the bodies were found to increase in size and to show nuclei which also were larger than normal. During the early stages of the disease the bodies were very minute and apparently increased in size with the progress of the disease. The outstanding characteristics, then, as he saw them, were that they appeared to grow, that they showed a structure like that of protoplasm, that they stained like protoplasm, and that they tended to be amoeboid in shape. Also, the tendency to cluster around the nucleus was comparable to the similar tendency among other known intracellular parasites such as the swarm spores of *Chrysophlyctis endobiotica* Schilb in the potato wart disease (Orton and Kern, '19). The bodies, therefore, in his estimation, had many of the characteristics of a living organism. However, they had never been cultured, and until that might be possible they could not be definitely considered as the causal agency.

The vacuolate bodies found here were entirely different from

the granular mass described by Matz ('19) in the yellow stripe disease of cane, since the latter did not stain like protoplasm, did not show a protoplasmic structure, were not vacuolate, did not contain any of the dark-staining granules, and were not plastic. Neither were they like the bodies associated with the Fiji disease of cane, nor like the plasmodia of *Plasmodiophora Brassicae*. They did, however, resemble remarkably the Negri bodies associated with rabies in the brain cells of the diseased dog.

Similar vacuolate bodies have been found in wheat plants infected with the rosette disease and the mosaic-like leaf mottling by McKinney, Eckerson, and Webb ('23a). They were found in the roots, crown tissue, leaf sheaths, and leaves, never necessarily occurring in close contact with the nucleus. Their contents were rather homogeneous in structure, and they contained many large and small vacuoles in which granules could be observed showing Brownian movement. No independent movement was ever observed in the living cells. From these observations they concluded that, although the bodies might be a stage of some definite parasite, yet their distribution in the host tissue and their apparent parallel development with that of the host cells did not seem to conform exactly with the distribution and development of any plant parasite known.

The same investigators (McKinney, Eckerson, and Webb, '23) have described similar bodies in the chlorotic areas of the leaves of *Hippeastrum Johnsonii* which were infected with the mosaic disease, as did also Kunkel ('24a) in *Hippeastrum equestre* Herb. Kunkel found them to be similar to those in corn as regards distribution, although the *Hippeastrum* bodies were considerably smaller. They were never found in tissues until the mosaic blotching appeared, and the size of the bodies was directly proportional to the degree of chlorosis, the lighter areas showing the largest bodies. Kunkel also found such amoeboid-like structures in the chlorotic areas of mosaic-diseased *Brassica pekinensis* (Skeels), which were similar to those in corn and in *Hippeastrum equestre* Herb. both in structure and in staining reactions. The *Brassica* bodies differed, however, in not being primarily associated with the nucleus. He then concluded, from all of his observations on the bodies in the Fiji disease of

sugar cane, in the mosaic disease of corn, in *Hippeastrum equestre* Herb., and in *Brassica pekinensis* (Skeels), that the amoeboid bodies associated with these diseases might represent only one stage in the life history of the causal organism, and that at another stage they might be so small and plastic that they could pass through the fine pores of a filter and escape detection under the microscope. If this were the case, they would probably become visible only after a certain period of growth within the cell of the host. For the time, however, he added, "we must be content with the knowledge that intracellular amoeboid organisms accompany the mosaic disease in several plants, that these bodies look like living organisms, and that in corn and *Hippeastrum* they are associated with chlorosis in such a way as to account for the mosaic pattern in the leaves."

Smith ('24) studied the leaf and stem tissue of varying age from mosaic-diseased potato plants and found in the chlorotic areas similar vacuolate bodies which showed definite walls and bore, as he said, "a superficial resemblance to some kind of protozoal organism." They were usually in close association with the nucleus of the host cell, and in the living cells they failed to show any automotive movement. In view of these facts and the further fact that in the light green areas in which these bodies were found the general disintegration of the tissue seemed to be considerable, he came to the conclusion that they were some type of degeneration product of the cell and probably of the nucleus, induced by the mosaic, and that they were the effects rather than the cause of the disease.

Contemporaneous with the studies on the mosaic disease and the associated amoeboid-like bodies in corn, sugar cane, potato, *Hippeastrum*, and *Brassica*, have been several notable contributions to a study of similar structures in the chlorotic areas of the mosaic disease in tobacco, tomato, and related genera. Dickson ('22), in observing living free-hand sections of the tobacco leaf, found in the chlorotic areas of leaves in the advanced stages of the disease, among the vacuolate bodies and the plate-like crystals, numerous smaller bodies exhibiting an erratic movement. He considered them as flagellates but in spite of careful staining he could obtain no proof of this. In fixed material he

found minute dark-staining bodies, .3 μ long and slightly less in width. They were found particularly in the border parenchyma of the vascular tissue of diseased leaves, but were also observed in close contact with the walls of the chlorenchyma cells and, in some cases, surrounding the chloroplasts. The vacuolate bodies, comparable to those described by Kunkel in corn, he believed were not the causal agency, but were, on the other hand, secondary in nature. He attempted to culture these very small bodies but was unsuccessful in isolating them from the virus. The plate-like crystals which have been described in connection with the mosaic disease of tobacco were believed by him to be due to a product of chlorophyll degeneration combined with changed plastid protoplasm, in view of the fact that Ewart had shown that CO_2, combined with chlorophyll in the presence of water to form xanthophyll and a colorless waxy substance,

In connection with these studies Dickson also made an anatomical study of numerous mosaic-diseased plants, from which he drew the following conclusions regarding the anatomical characteristics of the mosaic disease in general:

1. There was a difference in thickness between the chlorotic and the green area, the ratio being about 2:3, due to hypoplasia of both palisade and spongy parenchyma cells in the chlorotic areas.
2. The dark green areas exhibited hyperplasia.
3. There was a regular arrangement of cells in the light area, thus reducing the intercellular spaces.
4. The epidermal cells were smaller in area but deeper over the hypoplastic areas than over the normal.
5. Hypoplasia was accompanied by a degeneration of cell contents.
6. Disintegration of the chloroplasts was accompanied by the appearance of the very small hyaline bodies in rapid movement, the semi-crystalline plates believed to arise from degenerate chloroplasts, and the vacuolate bodies.

The vacuolate bodies in the hair cells of the mosaic-diseased tobacco were studied by Goldstein ('24) in the living condition. She found that in the chlorotic areas the hair cells and epidermal cells contained the vacuolate, more or less amoeboid bodies, and the crystals, both of which have been described and illustrated many times since their first description by Iwanowski ('03). However, she watched them and studied them more intensively

in the living condition than had been done heretofore. She found that the bodies bore no definite relation to the nucleus, but were simply in the cytoplasm and carried about in it when the protoplasm was streaming actively. Not only did she see the body carried around the cell in the protoplasmic streams but also she noticed an apparent change in form which might be described as an indication of automotive movement. In the pseudopodia of the more active bodies she occasionally observed a hyaline ectoplasmic-like cap bordered by a membrane. She interpreted her observations of such membranes as convincing evidence in favor of the view that the bodies are surrounded by a definite plasmatic membrane. Also, she found that treatment with acid caused the contents of the bodies to shrink, leaving visible the definite limiting membrane.

She mentioned the crystals but gave no results of her work on them, promising a paper in the near future. She did, however, treat the cells with various fixing fluids, watched the influence of these under the microscope, and found that the crystals lost their typical form, becoming long, irregularly lobed and striated masses which were stained deep yellow by the fixatives. This accounted for the appearance of the striate masses in the fixed mosaic tobacco tissues. She concluded from these observations that it might be possible that such plastic bodies as these would be able to pass through cell walls and the pores of bacterial filters just as the nuclei were observed to migrate from cell to cell in *Tradescantia* (Miehe, '01), thus explaining the nature of the virus.

Rawlins and Johnson ('24, '25) have studied cytologically the fixed tissue of the mosaic tobacco leaves, and have described three types of cellular inclusions,—the yellow-staining striate material radiating from the nucleus, small black-staining bodies, and vacuolate bodies varying in size from those just visible to those slightly larger than the nucleus. They found that the development of these inclusions was inhibited by temperatures which inhibited the expression of the mosaic. Also, it was observed that only 20 per cent of the plants showing the symptoms out of doors showed the bodies indoors, whereas 80 per cent of those in the greenhouse showed them. They have

attempted to show the sequence of the appearance of these various types of bodies. The first to appear was the striate material, the small dark-staining type, and the crescent-shaped vacuolate type, and they felt that the small bodies were a stage in the development of the vacuolate type. The crescent-shaped type definitely gave rise to the rounded vacuolate type which then persisted along with the striate material throughout the life of the leaf. They considered that the amorphous nature of the striate material indicated that it was a product of the diseased cell or of the causal agency.

Very recently Eckerson ('26) has described what she considered an organism of the tomato mosaic. She studied the tissues of tomato plants at various short intervals after inoculating the healthy plants and was able to observe at 24 hours after inoculation flagellate organisms in the veins and adjacent tissue, and the chloroplasts near the veins showed signs of dissolution. As the time after inoculation increased, the bodies became more numerous and the dissolution of the chloroplasts continued, while the bodies within them increased in size. Seven days after inoculation many of the chloroplasts in the palisade layer were in the process of liquefaction, while the remaining plastids contained non-motile bodies which seemed to be early stages of spore formation. Ten days after inoculation, when the leaflets began to show mottling, the palisade cells were partially disorganized, the cytoplasm was gone, the chloroplasts partially dissolved, and the remaining ones contained spores. These disorganized cells were usually bounded by groups of cells in apparently perfectly healthy condition. She has included many illustrations of both the spore and flagellate form, showing the nature of the organism. It is interesting that the organism should have been found associated with the chlorotic areas of mosaic-diseased plants, but she has not yet demonstrated that it is the causal agency by isolating it and inoculating the resultant culture into healthy plants. Moreover, it must be remembered that the size of the particle of the causal agency has been determined by Duggar and Armstrong ('23) through filtration experiments to be approximately the size of the particles in a fresh 1 per cent solution of haemoglobin, which is 30 μμ. The smallest size which Eckerson

gave for the flagellate forms she described is 2–4 μ, and this is approximately 1000 times the size of the particle as determined by Duggar and Armstrong.

The work of Nelson ('23) has not been included in this discussion, since it has been completely refuted by other investigators who have shown that the protozoan-like bodies which he described occur normally in the phloem tubes of healthy plants.

Structures similar to Nelson's bodies have been described by Klebahn ('26) in the sieve tubes of *Anemone nemorosa*, and he believes that evidence is strongly indicative of their being the cause of the disease called by him "alloiophylly." He considers that these bodies are closely related to the bacteria in that they show no definitely organized nucleus, and that their size seems to fall within the limits of the size of bacteria; and he applies the term *Scolecosoma anemones* to them. They have not, however, been found to reproduce by simple fission, and for this reason as well as the fact that they exhibit a great variation in form, they differ from the bacteria. He has, therefore, concluded that they are a "neuen Organismengruppe die etwa zwischen Bacterien und Flagellaten vermittelt." According to him, it is possible that his *Scolecosoma* and Nelson's bodies belong to the same or nearly related species, and it should still be an open question as to whether or not these belong to the same group of organisms as do the vacuolate bodies described as accompanying many of the mosaic diseases.

There have also been numerous cytological and physiological studies made on the chloroses of many of our variegated horticultural varieties. Although anatomical studies have been made, particularly by Funaoka ('24), there have never been found inclusions of the nature of those described as occurring in several of the mosaic-infected plants. However, the investigations have led to results so closely parallel to those obtained from work on the mosaic plants that a resumé of them would be of interest here in showing the possible connection between the two types of chloroses.

Masters ('69) considered albinism as a change due to the deficient formation of green coloring matter or chlorophyll. He distinguished between this condition and etiolation by the

fact that in the former chlorophyll seemed never to be formed in the affected parts, even if they were exposed to light, while an etiolated organ placed under favorable circumstances speedily assumed a green color. Later, Weiss ('78) explained variegations on the assumption that white spots were caused by the presence of air which was held in the intercellular spaces under the epidermis. He showed that leaves which were exhausted of air under water, by means of a pneumatic pump, lost the white spots. However, Dalitsch ('86) contributed the first accurate observations on the cause of variegation. He defined the white spots as due to chlorophyll-free cells in the fundamental tissue.

Saposchnikoff ('89) studied the starch content of chlorotic and green areas of variegated leaves and found starch only where chlorophyll was present. When, however, the leaves were placed in sugar solution, starch was found in equal amounts in the chlorotic and the green areas. In this connection, Winkler ('98), from a study of leucoplasts, chromoplasts, and chloroplasts, concluded that, whether the stroma was or was not stratified, whether it contained chlorophyll or some other pigment, whether the pigment was granular or crystalline, whether the plastid was large, small, distorted, or smooth, the stroma was always, when not too greatly reduced, able to form starch, if sugar were present.

Pantanelli ('05) made a physiological study of the variegations and found that it was possible to explain them on the basis of enzyme action. He found that the chlorotic areas were characterized by a decrease in chlorophyll content, increase in the accumulation of oxidizing enzymes, an increase in the osmotic pressure, lack of accumulation of mineral and organic salts and sugars, and a limitation of growth processes. His explanation of these observations is given in the following theory regarding the etiology of variegations. The first indication was probably an abnormal accumulation of the oxidizing enzymes, which disturbance probably took place in the stem or root; and then the disturbing material was carried up by material transport through the sieve tubes to the various parts of the plant. In the chlorenchymatous cells, it led to a destruction of the chlorophyll and to a general disease of the protoplasmic parts, which was evidenced by an increase in turgor. Further investigation

showed that the protoplasm and the plastids were disorganized and digested by the enzymes which were developed in abnormal quantities. The observed increase in osmotic pressure, he believed, was probably due to the increase in disintegration products of smaller molecular dimensions. He therefore considered that the pattern of the variegation followed the veins, through which the agency was carried and from which it was frequently distributed on one side only, making the vein the boundary between the green and the chlorotic areas.

Baur ('04) distinguished between the non-infectious and the infectious types of variegation, the former being transmitted through the seed, whereas the latter, although they could not be transmitted through the seed, could be passed on to healthy plants by grafting a variegated twig on a healthy stock. He found infectious variegation to be quite frequent among the *Malvaceae*, and he investigated in great detail the variegated variety *Abutilon Thompsoni*. A microscopic study of the leaf tissue revealed nothing of the nature of a causal organism, but only a reduction in the size and number of plastids and in the amount of chlorophyll contained by these in the chlorotic areas. In an attempt to determine the nature of the virus he tried many methods of transmitting it to healthy plants, but was successful only in the grafting experiments. In 1906, he concluded that the virus was not an organism but highly organized products of metabolism, which in a certain sense, possessed the power of growth. Such products passed through the cells of the plant, and in the embryonic cells of the young leaves they found free side-chains to which they attached themselves. In these cells, then, it was believed that the toxin was again formed anew. This physiological explanation is quite analogous to a similar explanation given by Hunger ('05) as the cause of the mosaic disease of tobacco.

A very similar theory was sponsored by Molisch ('08) in connection with his studies on *Abutilon Thompsoni*. He studied both living and stained material and found no structures which might be interpreted as living organisms. He also tried cultivating the virus on artificial media and on an extract of *Abutilon* leaves, but was entirely unsuccessful.

At this time, Kränzlin ('08) made a study of the pigments connected with variegated plants, and he obtained results which were confirmed later by the work of Colon ('19) in a study of the pigments associated with the mosaic diseases. These results indicate that the same pigments are present in both the chlorotic and the green areas, the difference being primarily one of quantity of pigment. In the chlorotic areas he found less of each of the normal leaf pigments, that is, he found a similar decrease in chlorophyll and in carotin. Colon, in his work, obtained similar absorption spectra from the two areas and concluded that the chlorotic nature of the spots in the yellow stripe disease of cane was not primarily due to a decomposition of the chlorophyll as such.

Küster ('19) found the veins incompletely developed in the chlorotic areas of the marginally variegated leaves of *Acer platanoides*, and hence concluded that degeneration or incomplete development of the green chloroplasts was probably caused by diminished nourishment. This was, however, refuted by Funaoka ('24), who studied the relative frequency of the veins in the chlorotic and the green areas of 14 species. The results showed that in 9 of the species there was an equal frequency, in 3 species there was a thicker network of veins in the chlorotic than in the green areas, whereas in a single species, *Euphorbia marginata*, the net was thicker in the green areas. In *Richardia Elliottiana* the vascular network was not developed in the chlorotic area. Hence, from his observations he concluded that in many plants the cause of variegation could not be traced back to an insufficient supply of vascular bundles and a resulting poor nutrition.

Funaoka also made an extensive study of 18 different variegations, and from his observations was able to classify them on the basis of their microscopic anatomical characters. The paper was well illustrated with semi-diagrammatic drawings showing that the chloroses might be due to a loss of chloroplasts in one or more layers of cells in the leaf (periclinal variegations), or to an anticlinal division of the green and white areas of the mesophyll, or a lack of differentiation of one or more layers, particularly the palisade layers. No indication of any structure resembling a microorganism was described.

From an investigation particularly of variegated varieties of *Zebrina*, Tsinen ('24) concluded that from the cytological point of view variegations occurred as the result of an alteration in the plastid mechanism of the cell. This alteration could take place before the differentiation of the plastids from the chondriosomes, at any stage during their development, or after they were mature, thus explaining the various types of variegations found.

Materials and Methods

Living and fixed materials were studied in plants infected with the mosaic disease and in the variegated plants. For a study of the living material the following vital stains were tried, —methylene blue, neutral red, bismarck brown, dahlia, and brilliant cresyl blue, the most favorable being the latter in concentrations of 1:10,000 and less. Thin sections of even the thinnest leaves, such as those of *Bougainvillea*, could be easily made by holding several pieces of the leaf within the same piece of pith, and then making free-hand sections of them. The sections were mounted in water and studied without any stain, after which a drop of brilliant cresyl blue solution, 1:10,000 was drawn under the cover-slip. The large vacuolate bodies, particularly those in *Petunia*, were found to stain very well after an exposure to the dye of from fifteen minutes to several hours.

For the fixed and stained materials, the following fixatives were tried,—Bouin's fluid (see Lee, '13, p. 65), Flemming's weak solution (see Chamberlain, '24, p. 25), medium chrom-acetic acid (see Chamberlain, '24, p. 25), osmic-sublimate mixture (see Lee, '13, p. 50), and an acetic-alcohol-formalin mixture (see Rawlins and Johnson, '25). In all cases small pieces of tissue were taken in order to insure rapid penetration.

These 5 different fixatives were tried on the tissue of tobacco, poke, and geranium mosaics, and on the variegations in *Evonymus japonica* Linn., *Ficus Parcellii* Veitch, *Ligustrum ovalifolium* Hassk., and *Abutilon pictum* Walp. in combination with each of the following stains,—Flemming's triple stain (see Chamberlain, '24, pp. 59–62), Delafield's haemotoxylin (see Chamberlain, '24,

pp. 45–48), and Haidenhain's iron alum haemotoxylin (see Chamberlain, '24, pp. 41–45).

The best results were quite generally obtained when the chrom-acetic acid was used as a fixative and was followed by the Haidenhain's iron alum haematoxylin. Counterstaining with the Orange G was found to be very desirable, since the vacuolate bodies seem to show a strong affinity for it. The most satisfactory method for introducing the counterstain was to dilute a 1 per cent solution of the stain in clove oil to a light amber color and place the slides in it for 10 to 15 seconds before placing them in xylol. This combination of chrom-acetic acid as a fixative, and Haidenhain's iron alum haematoxylin counterstained with Orange G was then used in all subsequent preparations, since it seemed the most generally successful.

With this technique, then, the following mosaic diseases were studied,—tobacco (*Nicotiana Tabacum* Linn.), petunia (*Petunia* sp.), columbine (*Aquilegia caerulea* James), pokeweed (*Phytolacca decandra* Linn.), and Jimson weed (*Datura Stramonium* Linn.). As to variegations, the following were chosen because they represent both Monocotyledons and Dicotyledons, because there are among them both infectious and non-infectious chloroses, and because they show quite different and distinct anatomical variations: *Homalomena cordata* Schott, *Ficus Parcellii* Veitch, *Nerium Oleander* Linn., *Coleus Blumei* Benth. var. "Mrs. Kirkpatrick," *Bougainvillea glabra* Choisy var. "*variegata*," *Pittosporum Tobira* Ait. var. "*variegatum*," *Evonymus japonica* Linn. var. *argenteo-variegata*, and *E. japonica* Linn. var. "*mediopicta*" Hort.

The cytological studies were made with a Zeiss microscope equipped with a 1/12a fluorite or semi-apochromatic objective, and a 4-mm. achromatic objective number 6. While studying the preparations the binocular tube was used with number 4 oculars, but all drawings, with the exception of text-figs. 2, 3, and 4, were made with the monocular tube, using a 12x compensating ocular. The text figures just mentioned were drawn with a 2x ocular and the 4-mm. objective. All drawings were made with the aid of a Spencer camera lucida.

Observations and Discussion

MOSAICS

Preliminary to the following work, a survey was made of living and fixed material of many of the mosaics at the author's disposal, with the idea of determining those which seemed most favorable for more intensive study. Several which are not mentioned here, such as *Crotalaria*, geranium, and poinsettia, have not as yet been sufficiently studied to warrant a report in the present paper, but it is hoped that the work on these may be forthcoming in the future. The studies given here have been made upon tobacco, *Petunia*, *Datura*, pokeweed (*Phytolacca decandra*), and *Aquilegia caerulea*.

1. Tobacco.—The work began with a study of the hair cells in the chlorotic areas of tobacco, since the inclusions here have been frequently described and since these cells seemed to offer such good material for the study of the bodies and crystals in living cells. Epidermal and hair cells were studied in the living condition, while in the case of fixed materials cross-sections of the leaves were used. In the living cells, irregular, vacuolate, amoeboid-like bodies together with clear plate-like crystals were found just as illustrated by Goldstein ('24). No indication of anything comparable to a nucleate structure was observed in either the fixed or living bodies, and they appeared to lack a limiting membrane of any sort. In the living cells, when protoplasmic streaming was sufficiently rapid both the bodies and the polygonal, flat, plate-like crystals were carried with it around the cell, but at no time could any movements which might be interpreted as automotive be discerned. The vacuolate bodies apparently changed their form somewhat, but all of these changes could be explained by the fact that the body was being turned over in the stream just as were the large crystals. There seemed to be no connection between the nucleus and the vacuolate bodies, the only times when they were adjacent to each other being when the nucleus acted as an impediment to the body as the latter was being carried through the cell in the protoplasmic stream. Both the bodies and the crystals were strikingly similar to those in *Petunia*, and since they will be described later, it is not necessary to go into detail at this point.

Rawlins and Johnson ('25) showed that in tobacco mosaic the bodies were found in 80 per cent of the mosaic plants grown under greenhouse conditions, whereas only 20 per cent of those grown outside exhibited them. In the present investigation it was also found that in the tobacco the inclusions were of more common occurrence in the greenhouse plants than in those grown out of doors. The idea arose that perhaps the ultra-violet rays which reach the plant when it is grown outside but which are cut out by the glass of the greenhouse might be, at least in part, the cause of this difference. With this idea in mind the following experiments were conducted.

EFFECT OF THE LONGER ULTRA-VIOLET RAYS ON TOBACCO PLANTS INFECTED WITH MOSAIC DISEASE

Ultra-violet rays may be divided into two diametrically opposed categories in regard to the action on living organisms. Those with the longer wave-lengths, 400–290 μμ (4900 A. U.–2900 A. U.) are commonly known as the biological rays. Since they are relatively penetrating, they include that range of the ultra-violet which may be present in the solar spectrum as it reaches the earth's surface. As to their action on living organisms, they are characterized by being chemically oxidizing and hence metabolic synergists. Opposed to this division, are those with the shorter wave-lengths which are commonly termed abiotic rays, because of their action on living protoplasm. In contrast with the longer rays, they are chemically reducing and metabolic depressors. They are so readily absorbed that penetration is very slight, hence they are never present in the solar spectrum as it reaches the earth's surface. In fact, the shortest wave-lengths obtained in the solar spectrum are about 291 μμ. All the shorter wave-lengths are absorbed by the earth's atmosphere. Because of the poor powers of penetration the abiotic rays are known to be superficial in action, being unable to penetrate the human epidermis. It is, then, recognized that the abiotic rays are lethal to bacteria and other living organisms, and these are concerned in sterilization processes.

Since the abiotic rays are so poorly penetrating and since it is the biological rays which have such profound reactions on the tissues of higher animals, it was the effect of these latter

rays on the tobacco plants infected with mosaic in which the author was primarily interested. In the experiments 2 types of lamps were used,—the Alpine Lamp, of the Hanovia Chemical Co., and the Air-cooled Quartz Mercury Vapor Lamp, of the Burdick Cabinet Co. Both the biological and the abiotic rays can be obtained from these lamps, depending on the distance between the burner and the object exposed. A column of air of 36–40 inches will absorb the abiotic rays, leaving only the longer biological rays. Therefore, to test the effect of the abiotic rays, the object is placed within 6 inches of the burner, whereas in an investigation of the longer wave-lengths, the object is placed at least 36–40 inches from the burner.

In the literature there has been very little experimental work on the effect of the biological ultra-violet rays on the tissues of plants, hence many difficulties arose in connection with the details of applying the lamp. The plants were found to burn most easily, particularly with the new Burdick lamp, so the time of exposure and the working distance (i. e., the distance between the burner and the plant) had to be determined for each lamp.

The best results were obtained with the Hanovia lamp, because it contained a very old burner in which the intensity of the rays had been decreased considerably. With this lamp it was found that the plants could be given a treatment of 30 minutes at 40 inches daily without bringing about fatal injuries to the plants, although they did become severely dwarfed.

Six plants were inoculated with filtered juice from the leaves of mosaic-infected tobacco plants, and the ultra-violet treatment was begun 2 days later, after the plants had recovered from the ill effects of the inoculations. They were rayed daily for 30 minutes at 40 inches, being kept, during the remainder of the day, under normal greenhouse conditions. At the end of 9 days all 6 plants showed the normal mosaic symptoms. With continued treatments the plants gradually became more and more dwarfed, and with the noticeable dwarfing the symptoms became less evident. At 20 days after inoculation the plants had apparently completely lost all of the typical mosaic symptoms. The treatment was continued for an additional 8 days, thus making the entire treatment 4 weeks in duration.

After treatments were stopped the plants were kept under observation in the greenhouse for 40 days, at the end of which time 2 of the 6 plants had re-developed the mosaic symptoms, 2 remained uniformly green, and the remaining 2 succumbed to the treatment which they had received. Therefore, the loss of symptoms after 18 days of exposure to the ultra-violet was apparently simply a masking of them, such as has been observed in plants which have been kept under blue light (Lodewijks, '10; Chapman, '17; and Dickson, '22). There was not a permanent curative effect. As has been noted, the plants were dwarfed, showing that the conditions to which they were subjected during treatment were not optimum for growth, and it is a well-known fact that the symptoms are the most prominent when the plants are growing rapidly. Therefore, it was not surprising that the disease was masked.

This masking of the mosaic symptoms in tobacco plants which were exposed to the biological ultra-violet rays would seem to substantiate the work of MacMillan ('23), in which he observed a masking of the mosaic symptoms in potato plants grown at high altitudes. He suggested at that time that the masking might be due to the biological ultra-violet rays which are more abundant at high than at low altitudes, and which, he believed, stimulated chlorophyll production in these cells which would be chlorotic under ordinary circumstances.

Pieces of the leaves were taken at random from the plants when the treatment was completed, and studied in fixed material as well as in the living condition. The vacuolate bodies and the crystals were still present in some of the hair cells, indicating that the ultra-violet rays were not the cause of the absence of the bodies in the mosaic plants which were grown out of doors. Little effect was found in the tissues other than a more uniform distribution of the plastids and a more constant differentiation of palisade and spongy mesophyll throughout the leaf of the rayed mosaic plants; likewise, in the latter there was an increase in the number of epidermal hairs over that in the control plants.

Experiments conducted in the same manner with the Burdick lamp gave comparable results, but in the case of this new burner the intensity of the rays was so great that the burning and

injury to the plants were difficult to avoid, even with exposures of only 1 or 2 minutes at a working distance of 50 inches. This again illustrates the great difference between burners and shows that the time and working distance of exposure must be determined for each burner with each type of plant. From these results it can be concluded that, in the case of the tobacco plants infected with mosaic, the only effect of the biological ultra-violet rays is the inhibition of normal growth, which in turn causes a masking of the mosaic symptoms. Moreover, the fewer inclusions in the cells of the plants grown out of doors than in the greenhouse plants cannot be explained as a result of the action of the biological rays of ultra-violet light.

EFFECT OF THE SHORTER ULTRA-VIOLET RAYS ON THE MOSAIC VIRUS

This study of the effect of the biological ultra-violet rays on the tissues of plants infected with mosaic suggested the idea of determining the effect of the abiotic rays on the virus itself. As has been explained, the shorter wave lengths are not penetrating and can be obtained only at the very short working distance of 6 inches. Hence, the effect of the abiotic rays on the virus cannot be studied by subjecting the plants directly to these for two reasons: first, the shorter wave-lengths are so poorly penetrating that they cannot pass through the epidermal cells; and, second, the plants so treated would be immediately burned and killed. In these studies, therefore, the effect of the rays on the virulence of the mosaic tobacco juice was investigated.

Fresh mosaic tobacco leaves were ground, the juice filtered through cotton, water added to make a 1 : 4 dilution, and this filtered through a spherical atmometer cup. This procedure removed from the juice the chlorophyll, which, if it had been present during the subsequent treatment, would probably have absorbed all of the shorter wave-lengths and a determination of their effect on the virus would have been impossible. The filtered juice, however, was a clear solution, thus avoiding the suggested difficulty.

Five-cc. portions of this clear, filtered mosaic juice were placed in uncovered 50-mm. petri dishes and then exposed to the ultra-violet rays at a distance of 6 inches for varying periods of time as

seemed desirable from the preliminary experiments. Ten plants were then inoculated with the rayed juice in the case of each exposure, and, as a control, 10 plants were inoculated with the filtered juice which had not been rayed. The experiments were run at 2 different times during the winter and in different compartments of the greenhouse, with similar results, thereby assuring that environmental factors were neither favoring nor masking the expression of the symptoms. The virulence of the virus was considered to be indicated by the percentage of plants showing the mosaic symptoms. Since preliminary experiments showed that the virus was in no way inactivated by an exposure of 5 minutes, exposures shorter than that were not repeated in these two series of experiments. In this work, the new Burdick lamp was used, operating at 8 amperes and 70 volts. The results are given in the following tables.

TABLE I

INFLUENCE OF ABIOTIC RAYS ON THE MOSAIC VIRUS

Length of Exposure	Results	
	1st series	2nd series
Control	9 affected in 9 days	10 affected in 11 days
5 minutes	8 affected in 9 days	10 affected in 12 days
10 minutes	5 affected in 9 days	6 affected in 14 days
20 minutes	1 affected in 9 days	2 affected in 14 days
30 minutes	None affected in 9 days	1 affected in 14 days

In the experiments the remainder of the plants continued healthy for a period of 5 weeks and were discarded at that time. These results show clearly that the virus has, with sufficient exposure to the abiotic rays, been permanently inactivated. The same data show that the process of inactivation has been gradual, since there is some reduction in the virulence of the virus with an exposure of 10 minutes. The exposure is not lethal, however, until the virus has been subjected to the rays for a period of 30 minutes. The single plant which succumbed to the disease in the exposure of 30 minutes in the second series may have been an accidental infection.

The nature of this inactivation is not understood. An explanation of this process would probably at least suggest the

nature of the causal agency. The inactivation is not of the same nature as the killing of bacteria by the abiotic rays, since the time required is of an entirely different order. In the inactivation of the mosaic virus, exposures as long as 30 minutes are necessary, whereas, so far as known, all micro-organisms are killed by exposures which are measured in seconds rather than minutes.

The writer tried the influence of the rays from this same burner on *Bacillus prodigiosus*, with the idea of comparing the killing time here with the inactivation time in the case of the virus. Transfers of the organism were made, and when the cultures were 48 hours old a suspension was made in sterile distilled water. Plates were poured which contained 1 cc. each of a 1 : 10,000 and 1 : 100,000 dilution of the original suspension, in order to determine the density of the suspension. Counts of these plates showed that the original suspension contained approximately 12×10^5 organisms per cc.

The original suspension and a 1 : 1,000 dilution of that suspension were then treated by the same method as was the virus in the above experiment, 5 cc. of the suspension being removed with a sterile pipette and placed in sterile petri dishes which were kept covered except during the short periods of exposure. Duplicate exposures were made in each case, and two plates inoculated from each exposure. The plates each contained a 1-cc. sample from the 5-cc. portion exposed to the rays. The results obtained are tabulated in table II.

TABLE II

EFFECT OF ABIOTIC RAYS ON BACILLUS PRODIGIOSUS

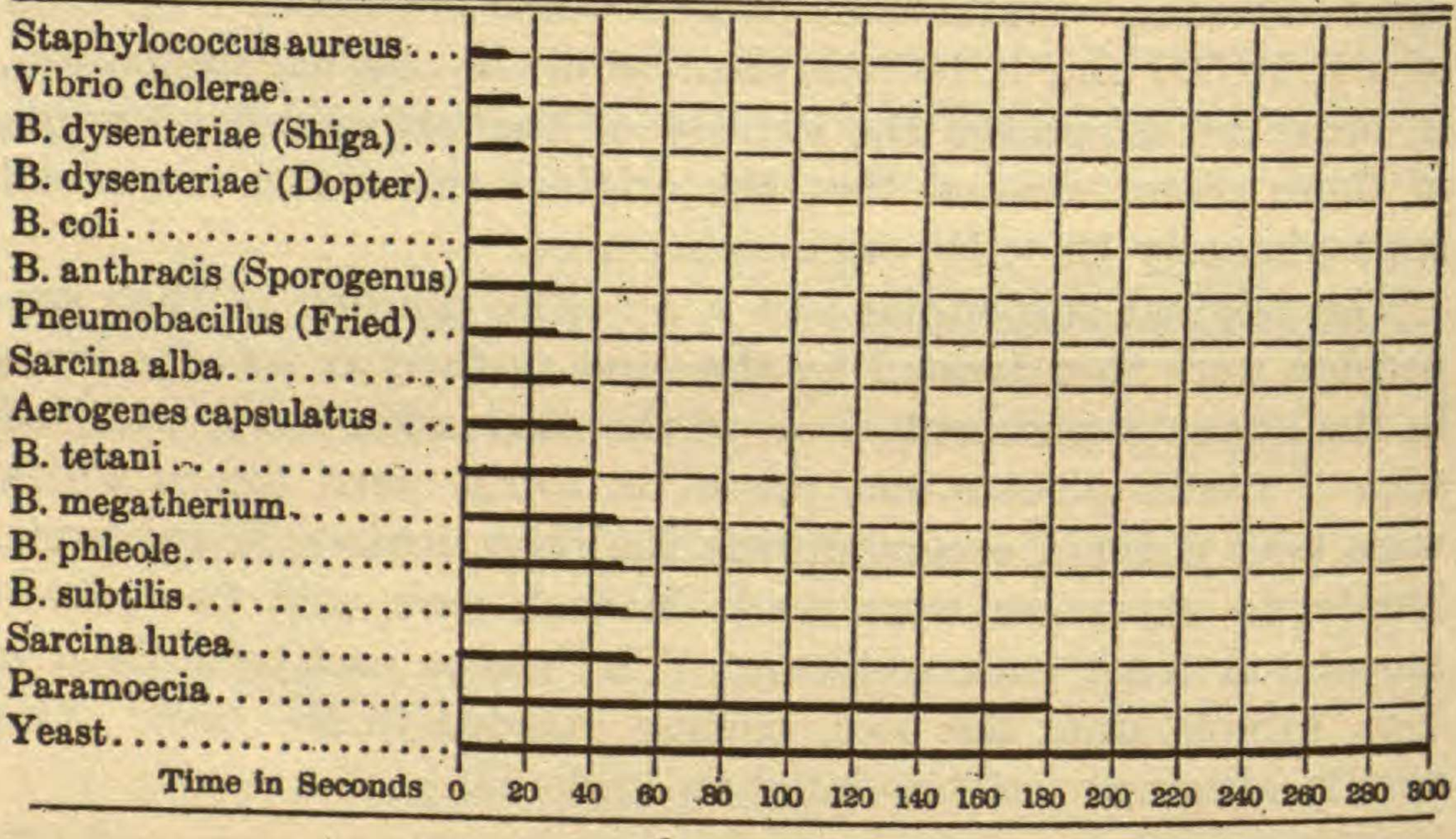
	Exposure			
	½ minute		1 minute	
	Series A	Series B	Series A	Series B
Orig. suspension	6 2	7 0	5 2	2 1
1 : 1,000 dilution	0 1	0 0	0 0	0 0

These results show that a suspension of *Bacillus prodigiosus*

of 12×10^5 per cc. can be practically killed by an exposure to the shorter ultra-violet rays given by the Burdick lamp when operated at 70 volts and 8 amperes. The killing time, which is 30 seconds in the case of *Bacillus prodigiosus*, therefore, is certainly not of the same order as the time of exposure required for inactivation of the virus. That this short killing time is a common characteristic of many organisms is shown in fig. 57 of Ellis and Wells ('25) which is included here as table III.

TABLE III

TIME IN SECONDS REQUIRED TO DESTROY VARIOUS ORGANISMS AT A DISTANCE OF 200 MM. FROM A QUARTZ MERCURY LAMP OPERATING AT 66 VOLTS AND 3.5 AMPERES



It is of interest to note from the table just referred to that the spores are not much more resistant to the abiotic effect of the rays than is the vegetative growth. According to Ellis and Wells von Recklinghausen has shown that while spores are 20 times more resistant than the vegetative forms to the action of chemical germicides, they are only 3 times more resistant to the ultra-violet rays than the vegetative forms.

These experiments show, then, that the abiotic rays can inactivate the virus if the latter is exposed to them for a sufficient length of time, but this time factor is many times greater than that which is necessary for the killing of the common micro-

organisms, either in spore or vegetative form. Hence, these results would seem to indicate that the virus is not an organism in nature. Whether the time factor is comparable to that which is necessary for the inactivation of enzymes has not yet been determined, and such determinations will give further indication as to the nature of the virus. The inactivation may simply have been due to a precipitation of certain proteins, since with the longer exposures a certain turbidity was observed in the formerly clear solution, and it is known that the rays will decrease the stability of the solution of some of the proteins, particularly the albumens.

2. Petunia sp.—A study of living free-hand sections of leaf tissues of healthy petunia plants and those infected with mosaic revealed in the latter the vacuolate bodies and the clear plate-like crystals similar to those observed so frequently in tobacco as studied particularly by Goldstein ('24). Just as in tobacco, the hair cells and the epidermal cells offered unusually favorable material in which to study the bodies in the living condition.

Although several leaves were studied from each of numerous healthy plants, no such inclusions were found. The normal petunia hair cell, as shown in pl. 13, fig. 1, contained only the nucleus, the cytoplasmic threads, and occasional small plastids carried along in the streams. The nucleus was found either suspended in the vacuole of the cell by the cytoplasmic threads, or closely pressed against the edge of the cell.

In the leaves of plants infected with mosaic, the hair cells in the dark green areas were similar to the healthy cells, showing no unusual inclusions. In the chlorotic areas, however, there were universally present both the vacuolate bodies and the plate-like crystals. Contrary to the distribution found by Goldstein in tobacco, there were never more than one or two of the vacuolate bodies present in a single cell at a given time. She illustrated as many as five in a single hair cell. The crystals, however, were present sometimes singly (pl. 13, fig. 2); sometimes as two or more separate and distinct crystals, each being carried about in the streams by itself; and sometimes in masses of numerous individuals lying adjacent to each other but not fused (pl. 13, fig. 19).

The cells were studied unstained as well as treated with various vital stains. The most successful vital stain employed was brilliant cresyl blue. The cells were mounted in water, and after they had been studied in the unstained condition a drop of a 1 : 10,000 solution of the stain was drawn in under the cover slip. The stain was taken up in from 15 minutes to 2 hours by the bodies, which could then be identified in the mesophyll, as well as in the epidermal hair cells.

The bodies exhibited different forms, varying from more or less definitely rounded, finely or coarsely granular structures, to irregular, vacuolate, amoeboid-like bodies. A limiting membrane was never observed except when the cells had been treated with 15 per cent alcohol and shrinkage had taken place, leaving visible the structure which was apparently a limiting membrane, as shown in pl. 13, fig. 9. The vacuoles varied in size, some bodies containing several large ones, and others numerous smaller ones giving them a porous or spongy appearance. They were not definitely associated with the nucleus as Künkel ('21) described them in corn, although at times they did appear adjacent to it. However, when the living cells were watched for a considerable length of time it was found that in those which were actively streaming the bodies were carried along in the streams. The nucleus frequently acted as an impediment in the course of the body, delaying its passage through the cell. If the cell had been observed only at that particular time the natural conclusion would have been that there was some association between the nucleus and the body, whereas a continued observation of the same cell showed that this was not the case. Never was one of these bodies seen in the process of division.

Single cells were watched for 2- and 3-hour periods of time, and the bodies and crystals observed as they were carried along in the protoplasmic streams. During such observations over long intervals of time, the bodies were seen to change in shape as well as to advance with the streams. The cell illustrated in pl. 13, fig. 2, was kept under observation for 2 hours, and during that time the body was perpetually changing form, as shown in the 40 camera-lucida sketches in text-fig. 1. The body apparently sent out short pseudopodium-like projections and these pro-

jections always appeared in the direction in which the body was being carried by the protoplasmic streaming. When meeting an obstacle such as the crystal, the nucleus, or some of the plastids, it would be stopped for a short time, then shape itself around the obstacle, and in a short time pass around it and continue to be carried in the stream. When reaching the end of the cell, the body could frequently be seen to flatten out in the stream against the cell wall and then again round up and continue its course back through the cell.

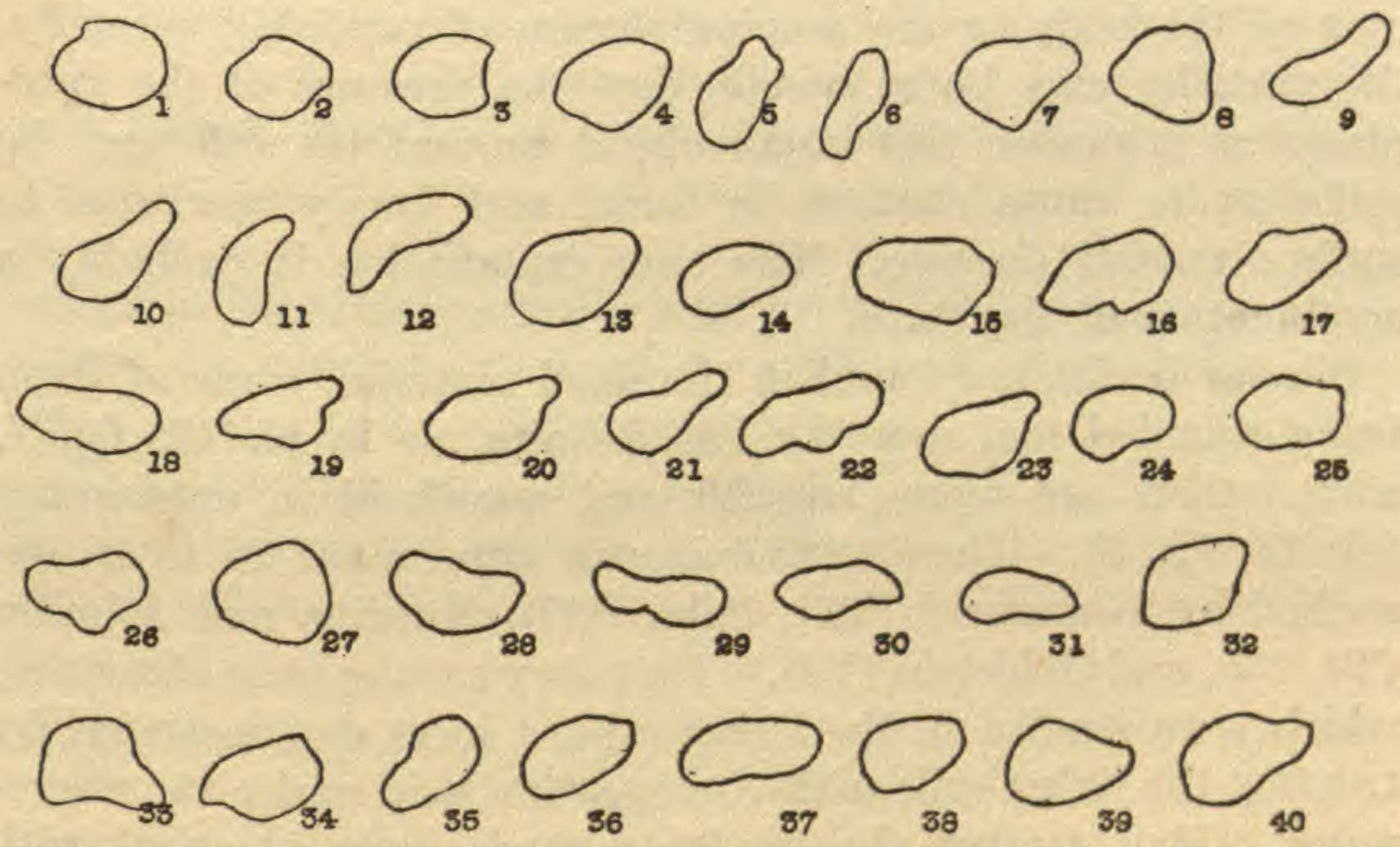


Fig. 1. Camera-lucida sketches showing changes in outline exhibited by the vacuolate body in the diseased hair cell of *Petunia* shown in pl. 13, fig. 2. These changes occurred during a two-hour period of observation while the body was being carried through the cell by the rapidly streaming protoplasm. × 1250.

At first, these movements were interpreted as being automotive, but further study seemed to show that they probably were not. If they were automotive, why did such movements occur only in those bodies in cells which were showing rapid streaming? They were never witnessed in cells unless there was a very pronounced cyclosis. Similar movements were observed by Goldstein ('24) in the bodies in the hair cells of tobacco and have been interpreted as sluggish amoeboid movements, but the author does not agree with her interpretation. If they were autonomous movements they should occur in the trichome cells independently of the streaming movements of the cyto-

plasm. Until further investigations have been conducted, such as attempting to remove the bodies from the cells by micro-dissection, it is impossible to state whether or not these bodies have automotive powers. At present, however, the author favors the view that the bodies are not the causal agency, but rather the result of a reaction between the cytoplasm and the virus. The apparent autonomous movements may have one of the 3 following explanations, or a combination of them. In the first place, these apparent changes may be due to the rolling over of the body in the moving stream of particles; secondly, the particles may be so mobile that the pressure of the cytoplasm as it pushes this larger object around the cell may be sufficient to cause changes in form, and finally, perhaps, to surface tension changes. The true explanation is probably a combination of the three.

Similar bodies are found in the fixed material, some of them being rounded and more or less definite, as in pl. 13, fig. 7, while others are more irregular and amoeboid in appearance (pl. 13, fig. 8). These are similar in appearance to those described by Iwanowski ('03), Palm ('22), Rawlins and Johnson ('24, '25), and Goldstein ('24). That they are the same structures which were studied in the living cells, I have demonstrated by treating the living cells with chrom-acetic acid under the microscope. Thus treated the fixed structures were identical with the bodies in the living cells.

The plate-like, highly refractive crystals were also studied. These, too, were observed to be transported in the cytoplasmic streams, but usually not as rapidly as the smaller bodies. As they were turned over by the pressure of the rapidly streaming cytoplasm, they showed various appearances, sometimes appearing long and narrow when viewed on edge; often appearing almost ribbon-like when viewed at an angle; and at other times as the flat plate-like structures shown in pl. 13, figs. 2 and 19. Their nature has not been determined, although the effect upon them of various solvents has been tried. They are definitely associated with the chlorotic areas of the leaves of plants infected with mosaic. They are not the result of a precipitation of products in the cell-sap, because they are definitely carried in the cyto-

plasmic streams. When studied in the living cells of tobacco by Dickson ('22) they were interpreted as being the product of chlorophyll degeneration combined with changed plastid protoplasm. His interpretation was based on the suggestion of Ewart's that CO_2 combined with chlorophyll in the presence of water to form xanthophyll and a colorless waxy substance. It seems improbable, however, that this is the explanation of the crystals obtained here, since in the epidermal and trichome cells in which they were studied the chloroplasts were few in number and very small in both the healthy and mosaic plants.

In the fixed material the crystals were not well preserved but were either striated in appearance or completely dissolved. These striated structures were observed by Iwanowski ('03) and interpreted as being plates of bacteria which were not visible except as the clear plate-like crystals in the living tobacco cells. As yet, no satisfactory explanation has been given of them, and there is none to be offered at present.

With the view of getting some indication of the nature of the bodies and the crystals in petunia, the solvent action of various substances, such as alcohol, formalin, KOH, HCl, HNO_3, and CH_3COOH were tried with the following results.

EFFECT OF ALCOHOL ON THE INCLUSIONS FOUND IN THE PETUNIA LEAVES INFECTED WITH MOSAIC

Free-hand sections of the chlorotic areas were made and studied in water mounts, thus insuring the presence of bodies and crystals. The sections were then placed in small vials containing approximately 1 cc. of the solvents, and examined at more or less frequent intervals during a 12-hour period. The solubility of the vacuolate bodies in the various dilutions was entirely different from that of the crystals, both products, however, being soluble in 95 per cent alcohol.

These results show that the bodies are soluble only in 95 per cent ethyl alcohol, whereas the crystals are attacked in as low as 10 per cent dilutions. Plate 13, figs. 9–12 inclusive, show bodies in cells which have been in 15, 30, 50 and 70 per cent alcohol for 12-hour periods, showing that they are not dissolved at those concentrations. When attacked by the alcohol, the crystals were seen to swell and eventually burst and become uniformly

TABLE IV

EFFECT OF DILUTION OF ALCOHOL ON SOLUBILITY OF INCLUSIONS

Solvent	Bodies	Crystals
C_2H_5 OH 5%	Not affected	Not affected in 12 hrs.
C_2H_5 OH 10%	Not affected	Dissolved after 6 hrs.
C_2H_5 OH 15%	Slightly plasmolyzed	Dissolved immediately
C_2H_5 OH 30%	Not affected	Dissolved
C_2H_5 OH 50%	Not affected	Dissolved
C_2H_5 OH 70%	Not affected	Dissolved
C_2H_5 OH 95%	Dissolved	Dissolved

distributed throughout the cell. This swelling took place immediately in all dilutions down to 10 per cent, and in this latter case the crystals were not completely dissolved until they had been kept in the solution for 6 hours.

EFFECT OF FORMALIN ON THE INCLUSIONS FOUND IN PETUNIA LEAVES INFECTED WITH MOSAIC DISEASE

Both the bodies and the crystals were found to be fairly resistant to the action of formaldehyde in 4, 8, and 12 per cent concentrations, the following results being obtained.

TABLE V

SOLVENT ACTION OF VARYING CONCENTRATIONS OF FORMALIN ON INCLUSIONS

Solvent	Bodies	Crystals
Formalin 4%	Not affected	Not affected immediately, but some disintegration after 12 hrs.
Formalin 8%	Not affected	Show signs of disintegration
Formalin 12%	Some disintegration	Some disintegration

The disintegration accompanying the treatment of the cells with 12 per cent formalin was not specific for the inclusions alone, since there was also a general disintegration of the cell contents. Plate 13, fig. 19, shows a portion of a cell containing a mass of crystals and a single body which had been in 4 per cent formaldehyde for 1 hour. All of the inclusions were still perfectly normal in appearance, showing a resistance to the solvent action of formalin. After having been exposed to the same solvent for 12 hours, however, the crystals began to exhibit the effects of the solvent action, losing their angular corners, becoming rounded off, and occasionally showing signs of the dissolution of the crystal,

as in pl. 13, fig. 18. Results comparable to these were obtained at the end of 1 hour with 8 per cent formalin, as is shown in pl. 14, fig. 33. On the whole, however, one may conclude that the bodies and the crystals are relatively resistant to the solvent or disintegrating action of formaldehyde.

EFFECT OF ACIDS AND ALKALIS ON THE INCLUSIONS FOUND IN PETUNIA LEAVES INFECTED WITH MOSAIC DISEASE

To determine the effect of an alkali, KOH was used in concentrations ranging from .25 to 4 per cent. In all cases all of the inclusions were attacked immediately, the cell contents being rendered almost homogeneous, except in the case of the .25 per cent solution. In this dilute concentration dissolution was not immediate, but gradual. All of the inclusions, however, disappeared within 6 hours.

Diametrically opposite results were obtained regarding the solvent action of the mineral acids, HCl and HNO_3. With concentrations as high as 10 per cent neither the bodies nor the crystals were injured, although the cells were severely plasmolyzed, as is shown in pl. 14, fig. 34. The results with acetic acid, however, were entirely different, the bodies not being injured by any concentration up to 10 per cent, whereas the crystals were dissolved in 20 minutes in all concentrations above 1 per cent, and in that concentration they were also dissolved in 1½ hours. These results of the effect of acids have been tabulated in table VI.

TABLE VI

SOLVENT ACTION OF ACIDS ON INCLUSIONS

Solvent	Bodies	Crystals
HCl 5%	Not affected	Not affected
HCl 10%	Not affected	Not affected
HNO_3 10%	Not affected	Not affected
CH_3COOH 1%	Not affected	Dissolved in 1 hour
CH_3COOH 2%	Not affected	Dissolved in 20 minutes
CH_3COOH 4%	Not affected	Dissolved in 20 minutes
CH_3COOH 5%	Not affected	Dissolved in 20 minutes
CH_3COOH 10%	Not affected	Dissolved in 20 minutes

From these studies, it can be concluded that the vacuolate bodies resemble the crystals in being relatively resistant to formalin, HCl, and HNO_3, and soluble even in .25 per cent

KOH. They differ, however, in their solubilities in alcohol and acetic acid, the crystals being dissolved in 10–95 per cent concentrations of alcohol and in 1–10 per cent concentrations of acetic acid, whereas the bodies are soluble only in 95 per cent alcohol and never in acetic acid. It is hoped that further studies in this direction and in micro-dissection may reveal something more fundamental regarding the nature of both the bodies and the crystals, together with an explanation of their connection with the chlorotic areas in mosaic-infected petunia and tobacco plants.

3. Datura Stramonium Linn.—Healthy leaves of *Datura*, together with those of plants infected with mosaic, were studied in material which was fixed in chrom-acetic acid and stained with Haidenhain's iron alum haematoxylin and Orange G. Leaves of approximately the same age were taken from the healthy and the mosaic-infected plants from which the histological and cytological studies here reported were made. It was found in the histological studies that neither the green nor the chlorotic areas of the mosaic-infected plants are comparable to the normal, the green being thicker than the healthy but showing normal tissue differentiation and distribution of plastids; whereas the chlorotic areas are approximately equal in thickness to those of the healthy but have poorly differentiated palisade tissue and show a decrease in the number of plastids. These differences are brought out in text-fig. 2, and the measurements are given in table VII. The figures given in table VII are in each case the average of 50 measurements made on numerous sections of different pieces of fixed material.

TABLE VII

COMPARATIVE MEASUREMENTS OF TISSUES IN HEALTHY AND MOSAIC INFECTED DATURA

	Healthy	Green area of mosaic	Chlorotic area of mosaic
Thickness of leaf	107.2 μ	208.9 μ	124.7 μ
Thickness of upper epidermis	10. μ	15.8 μ	19.4 μ
Thickness of palisade	48.9 μ	96.0 μ	40.3 μ
Thickness of spongy mesophyll	40.3 μ	75.6 μ	48.1 μ
Thickness of lower epidermis	10. μ	19.4 μ	17.1 μ
No. of rows of palisade cells	1.	1.	1.
No. of rows of spongy mesophyll	4–5	5–6	3–4
Longest diameter of chloroplasts	4.4 μ	5. μ	4. μ

A comparison of the measurements for the dark green area with those of the chlorotic area shows that the thickness of the leaf in the dark green area is 1.68 times as great as that in the chlorotic region; the palisade tissue is 2.38 times as thick; and the spongy mesophyll only 1.56 times as deep. This shows that the difference between the dark green and the chlorotic areas of the mosaic-infected plants is greatest in the palisade layer.

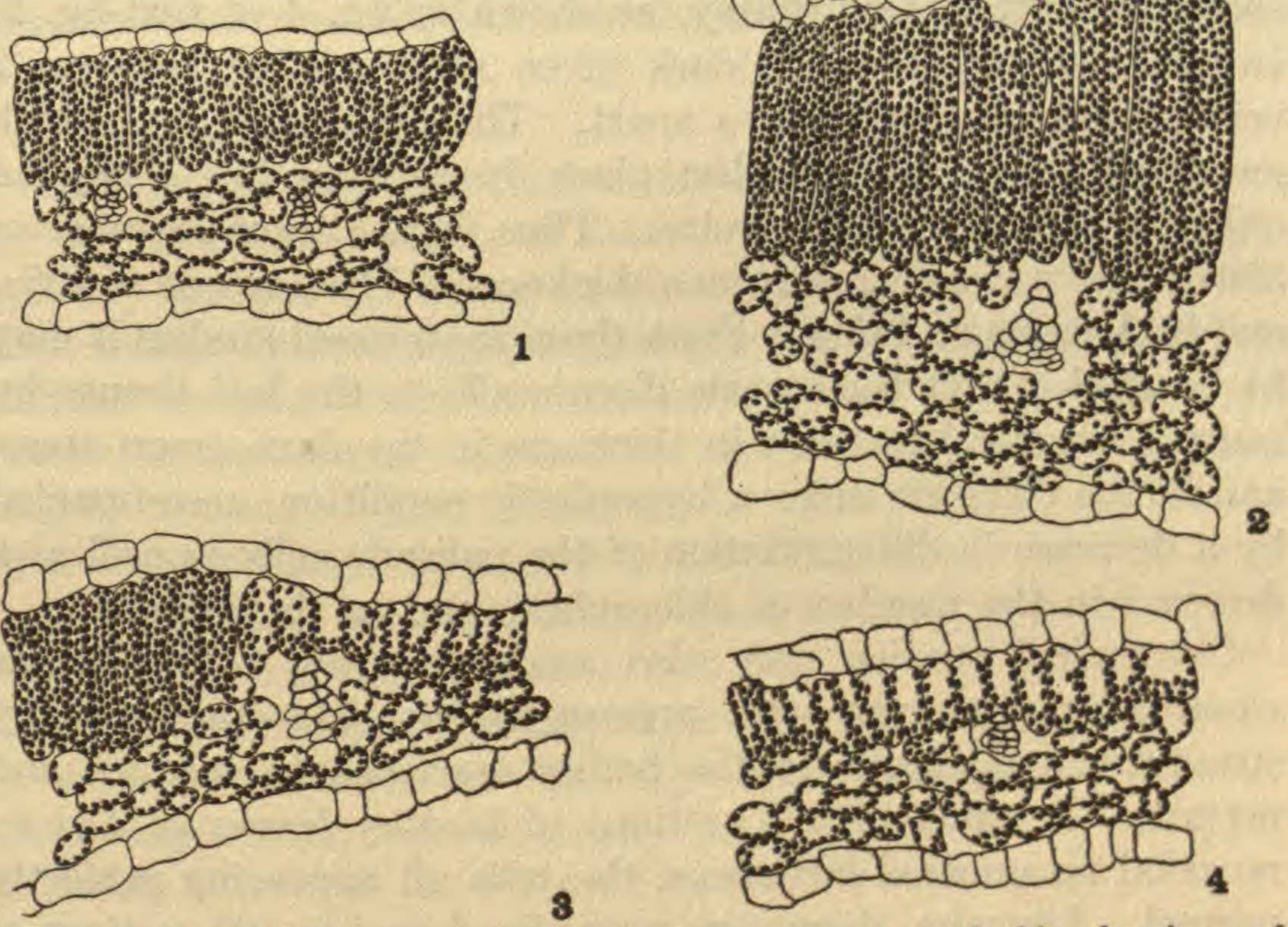


Fig. 2. *Datura Stramonium.* Semi-diagrammatic camera-lucida drawings of cross-sections of leaves, showing the effect of the mosaic on the tissues. × 300.
1, healthy leaf; 2, dark green area of a mosaic-infected leaf; 3, transitional area between the chlorotic and dark green areas of a mosaic-infected leaf; 4, chlorotic area of a mosaic-infected leaf.

This poor differentiation is shown more clearly in figs. 2, 3 and 4, of text-fig. 2.

On the other hand, a comparison of the measurements for the dark green areas of the mosaic-infected plants with those for the normal leaves shows that the increase in size of the dark green mosaic tissue over that of the healthy is approximately equal for all parts of the leaf. The thickness of the mosaic leaf in the dark green region is 1.96 times that of the healthy leaf; the upper epidermal cells are 1.94 times as deep; the palisade

layer is 1.96 times as high; the mesophyll layer is 1.88 times as thick; and the lower epidermis is 1.58 times as thick. Hence the dark green area, although exhibiting an enhanced thickness, shows no abnormal tissue differentiation. The plastid distribution, also, is similar in the dark green area of the mosaic-infected leaves and the healthy tissue.

In the diseased leaves, the transitional areas between the chlorotic and the dark green regions are very sharp, and apparently follow the veins closely, as shown in fig. 4 of text-fig. 2. In one case, there was a dark green area existing between 2 veins which were only 350 μ apart. The dark green area which measured 205 μ in the widest place dropped to 115 μ beyond either of the two limiting veins. Thus, in the short distance of 350 μ there was a change from a thickness of 115 μ to one of 205 μ and back again to 115 μ. From these anatomical studies it may be concluded that the mosaic disease affects the leaf tissues by causing a general increase in thickness in the dark green areas, and in the chlorotic areas a hypoplastic condition, accompanied by a decrease in differentiation of the palisade cells as well as a decrease in the number of chloroplasts and in their size.

Cytological studies were also made in order to determine whether or not there were present in the chlorotic cells any structures comparable to the bodies described in tobacco and petunia. A study of 100 sections of healthy leaves of *Datura* revealed no unusual inclusions, the cells all appearing perfectly normal. Likewise, there were no bodies found in 100 sections of the dark green areas of leaves of diseased plants. However, a study of the chlorotic areas revealed certain more or less irregular and indefinite structures, particularly in the upper epidermal cells.

These bodies resembled those described by Kunkel in corn much more nearly than they did those which are present in tobacco and petunia. They showed a strong affinity for Orange G, and at first sight gave the appearance of cytoplasm precipitated around the nucleus. Further study, however, demonstrated that they were a type of intracellular inclusion found only in the chlorotic areas of the mosaic-infected leaves. They were always adjacent to or surrounding the nucleus, showed

nothing of the nature of a limiting membrane, and were irregular in shape, size, and outline. Usually they were uniformly granular as in pl. 13, fig. 13, but occasionally they were more vacuolate as in pl. 13, fig. 15. In the vacuolate bodies, dark blue-staining granules could be observed as in fig. 15. Structures, such as the one illustrated in pl. 13, fig. 16, were apparently young stages in the development of the bodies, being small, taking the stain very lightly, and showing a tendency to be vacuolate. An unusual condition was found in which the nucleus was completely surrounded by the body, which last in turn was attached to the cytoplasm (see pl. 13, fig. 17).

At the time when the studies on the fixed material were made, no living diseased plants of *Datura* could be found, and consequently studies on living material have not yet been made. The author, however, feels that the bodies described here are definite entities which are associated with the chlorotic areas, since they were found only in those regions. When fresh material can be obtained observations will be made in order to see the appearance of these structures in the living cells.

4. Phytolacca decandra Linn.—Healthy and mosaic-infected poke were studied both in fixed material and in the living condition, the anatomical investigations being conducted as were those in the leaves of *Datura*. The following figures in each case are the average of 50 measurements which were made on the fixed material.

TABLE VIII

COMPARATIVE MEASUREMENTS OF TISSUES IN HEALTHY AND MOSAIC-INFECTED POKEWEED

	Healthy	Green area of mosaic	Chlorotic area of mosaic
Thickness of leaf	148 μ	191.5 μ	117.5 μ
Thickness of upper epidermis	15 μ	16. μ	15.5 μ
Thickness of palisade	57 μ	64.4 μ	27.3 μ
Thickness of spongy mesophyll	68.5 μ	100.2 μ	64.2 μ
Thickness of lower epidermis	7.5 μ	10.9 μ	11.0 μ
No. of rows of palisade cells	1.	1.	1.
No. of rows of mesophyll cells	±4.	±4.	4.
Longest diameter of chloroplasts	5. μ	5. μ	5. μ

Here, as in *Datura*, it can be seen that the mosaic disease modifies the entire leaf, the dark green area being thicker than

normal and the chlorotic region being reduced, particularly in the palisade layer. In this case also the healthy leaf is thinner than the dark green mosaic area but thicker than the chlorotic region. The thinness of the chlorotic sections is due chiefly to a lack of differentiation of the palisade layer, the cells of this last being 2.36 times thicker in the dark green than in the chlorotic regions whereas the mesophyll is only 1.71 times as thick. The chloroplasts are greatly reduced in number, particularly in the palisade layers, but their average size is not modified. As shown

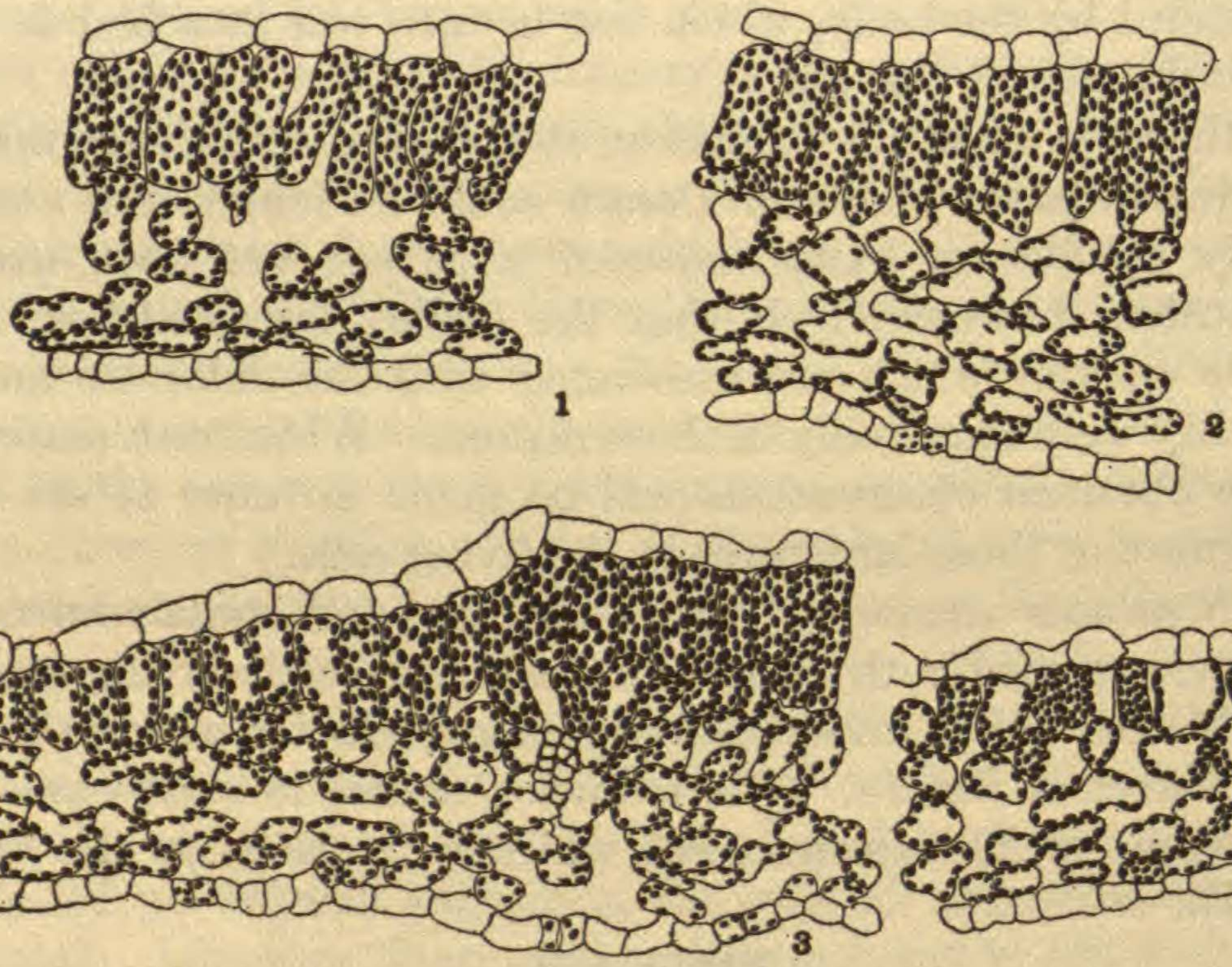


Fig. 3. *Phytolacca decandra.* Semi-diagrammatic camera-lucida drawings of cross-sections of leaves, showing the effect of the virus on the tissues. × 300. 1, healthy leaf; 2, dark green area of a mosaic-infected leaf; 3, transitional area between the chlorotic and dark green areas in a mosaic-infected leaf; 4, chlorotic area of a mosaic-infected leaf.

in fig. 3 of text-fig. 3, the transitional area is gradual and not sharp as it is in *Datura*, and the mottling may or may not follow the veins.

In addition to the anatomical study, cytological studies were made in order to locate any inclusions in the mosaic cells which were not in the healthy leaves. Very rarely bodies were found in the chlorotic areas, and although they were not of as common occurrence as in the other mosaics studied they were never

found in healthy leaves nor in the dark green areas of the diseased plants. Figs. 21–24 of pl. 14 illustrate the types of bodies found.

One type of structure found was that shown in the epidermal cell in pl. 14, fig. 21. Here the body was very definite in outline, stained orange-brown, and filled with numerous very small vacuoles, some of which contained dark blue-staining granules. There seemed to be no connection between it and the nucleus in this cell or in any other cell in which similar bodies were found. This, however, was not the typical kind of structure.

The more typical bodies were similar to the one in pl. 14, fig. 23. Here, the body took a faint blue-gray stain, contained several large vacuoles, and showed no limiting membrane. All such bodies appeared rounded rather than amoeboid in shape, and were but little denser than the surrounding cytoplasm, taking, however, a slightly different stain. In the other plants infected with mosaic disease, these vacuolate bodies showed a definite affinity for the Orange G, but in poke the blue-gray color indicated that there was some affinity for the haematoxylin. Such inclusions as these were more frequent than the others. In one case there appeared a very unusual modification of this type, as shown in pl. 14, fig. 22. Aside from its unusual shape, it was similar in all respects to the vacuolate bodies such as the one illustrated in pl. 14, fig. 23. Such bodies were found both in the epidermal and spongy mesophyll cells, but because of their poor staining reactions were sometimes difficult to study.

Figure 24, of pl. 14, shows a most unusual structure. It was very definite in outline, apparently being composed of 5 distinct component parts. It was definitely walled and took a differential stain, the periphery appearing orange and the central portion of each component part staining blue. This may have been an artifact, but it seemed too definite and it was thought to be of sufficient interest to be included here.

These structures were not abundantly distributed throughout the cells, but inasmuch as they were found only in the chlorotic areas, 100 sections of healthy leaves and a similar number of sections of dark green areas from mosaic-infected plants revealing nothing of the sort, it seemed of interest to include them here since they possibly accompany the chlorotic areas in mosaic-infected poke leaves.

5. Aquilegia.—Since 1919 Duggar has been observing a mosaic infection on plants of *Aquilegia* at the Missouri Botanical Garden. The diseased plants develop the typical mosaic symptoms including leaf mottling (see pl. 16, fig. 46), dwarfing of the plant, and decreased flower production. His inoculation experiments have indicated that it can be more or less readily transmitted. The infection was found on *Aquilegia caerulea* James, and since none of the healthy material of this species could be found, leaves of the hybrid *A. canadensis* Linn. × *A. californica* Gray were used for comparison with the plants infected with mosaic. Anatomical studies were made as in *Datura* and in pokeweed, and the measurements tabulated in a similar manner, as follows:

TABLE IX

COMPARATIVE MEASUREMENTS OF TISSUES IN HEALTHY AND MOSAIC-INFECTED AQUILEGIA

	Healthy	Green Area of mosaic	Chlorotic area of mosaic
Thickness of leaf	110 μ	150 μ	100 μ
Thickness of upper epidermis	15 μ	20 μ	20 μ
Thickness of palisade	42.5 μ	50 μ	29 μ
Thickness of mesophyll	38.5 μ	57.5 μ	32.5 μ
Thickness of lower epidermis	15 μ	20 μ	20 μ
No. of rows of palisade cells	2	2	1
No. of rows of mesophyll cells	±4	±4	±4

These measurements, together with the semi-diagrammatic drawings in text-fig. 4, show that the most striking difference between the chlorotic and the dark green areas is a loss of one of the palisade layers in the former; although there is also a decrease in the thickness of the mesophyll, that in the dark green area being 1.76 times as great as that in the chlorotic sections. Here, again, the healthy leaves are thinner than the dark green areas and thicker than the chlorotic regions. The chloroplasts show a gradual degeneration in the transitional areas between the dark green and chlorotic areas, as shown in fig. 3 of text-fig 4. This loss of an entire palisade layer in the chlorotic areas is similar to the condition described by Funaoka ('24) in some of the variegations, e. g., that in *Richardia Elliottiana*, and *Euphorbia marginata*, in which there is a loss of certain layers of tissue in the chlorotic areas. The chlorotic appearance is therefore due

to the absence of one of the palisade layers as well as to a decrease both in the number of plastids and the chlorophyll content of these.

Cytological studies were also made on the living and the fixed material of both healthy and mosaic-infected leaves, but, although 100 sections were studied of each, there were no inclusions of any

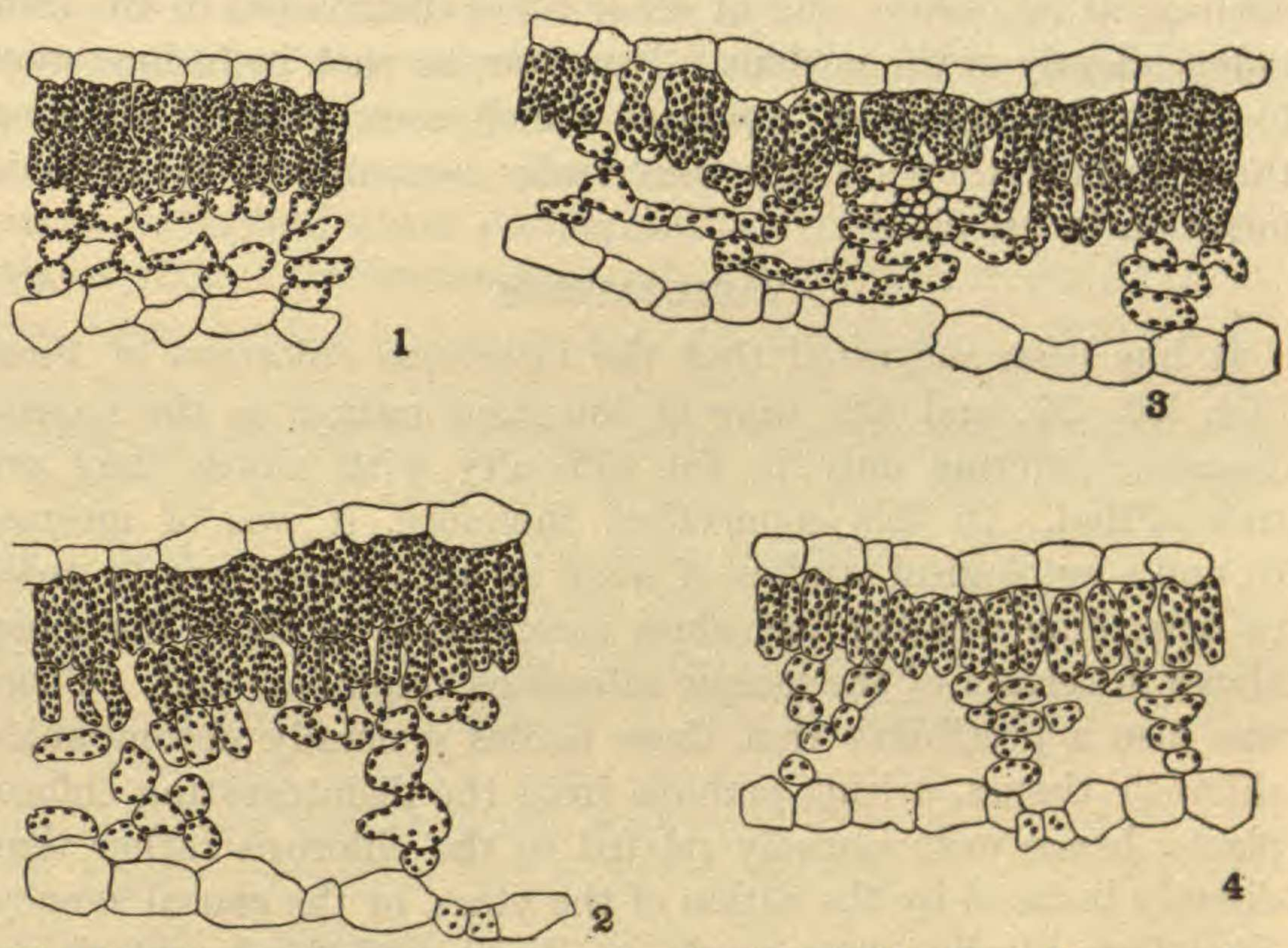


Fig. 4. *Aquilegia.* Semi-diagrammatic camera-lucida drawings of cross-sections of leaves, showing the effect of mottling on the tissue. × 300.
1, *Aquilegia californica* × *A. canadensis*, healthy leaf; 2, *A. caerulea*, dark green area of an infected leaf; 3, *A. caerulea*, transitional area between chlorotic and dark green areas of an infected leaf; 4, *A. caerulea*, chlorotic area of an infected leaf.

sort found in the chlorotic areas which did not also exist in the healthy and in the dark green portions.

From these studies on the tissues of healthy and mosaic-infected plants of *Datura*, pokeweed, and *Aquilegia*, it has been shown that the virus causes 2 changes in the leaves. First, it apparently stimulates certain areas to a general increase in thickness, and second in other sections of the leaves it causes a marked inhibition of growth, the reduction being chiefly in the palisade cells, and in the number and size of plastids, and the amount of chlorophyll which they contain. The transitional

areas may follow the veins and be fairly sharp, as in *Datura*, or they may not be necessarily associated with the vascular system, at which times they are very gradual, as in pokeweed. Associated with the chlorotic areas in tobacco, petunia, and *Datura*, possibly also in pokeweed, there were certain irregular vacuolate or granular bodies which at times were associated with the nucleus, as in *Datura*. and at other times distributed in the cells independently of the nucleus. However, no such inclusions were found in the tissues of *Aquilegia* which were studied, showing that perhaps they do not universally accompany the mosaic infection in all plants.

VARIEGATIONS

It has been suggested that the infectious chloroses of Baur ('04, '06, '07, and '08) were of the same nature as the mosaic diseases, differing only in the difficulty with which they are transmitted. In this connection, therefore, it was of interest to make cytological studies of some of his variegations in order to determine whether inclusions comparable to those described above in certain of the mosaic infections could be found. There was also a possibility that these bodies generally accompanied chlorotic tissues, arising perhaps from the disintegrating chloroplasts, hence were causally related to the chlorosis rather than directly induced by the action of the virus, or the causal agency. Therefore, studies were made on living and fixed material of various non-infectious variegations as well. A preliminary survey was made of many of those which were at the author's disposal, and from these the following were selected for more intensive study because they represent families in both the Monocotyledons and the Dicotyledons, because there are among them both infectious and non-infectious chloroses, and because they exhibit very different and distinct anatomical variations which, in some cases, are entirely different from those found in the mosaic plants infected with mosaic which have been studied.

1. Homalomena cordata Schott.—The variegation in this species consists of completely chlorotic round spots which, from microscopic observations, do not follow the veins, as shown in pl. 15, fig. 44. Free-hand sections were studied in the living condition, as well as sections of material which had been killed in chrom-

acetic acid, sectioned, and stained with Haidenhain's iron alum haematoxylin and Orange G.

Anatomical studies showed no difference in tissue differentiation between the green and the chlorotic areas. In neither region were the palisade cells developed, the leaf sections merely consisting of the upper epidermis, approximately 6 layers of spongy mesophyll, and the lower epidermis. The chief difference was the number and size of the chloroplasts; in the green areas, they were exceedingly large (averaging 8 μ in the longest diameter) and were more abundant, particularly in the upper layer of the spongy mesophyll which corresponds to the palisade layer in the ordinary leaf. On the contrary, the chloroplasts in the chlorotic areas were smaller and more sparsely distributed. The transitional region between the two areas was not sharp, the decrease in number and size of the plastids being very gradual. The nuclei in both the green and the chlorotic sections were strikingly large, both in the living and fixed materials.

Cytological studies, however, revealed no inclusions comparable in any way to those found in the plants infected with mosaic, although 100 sections of the fixed tissues and many living free-hand sections were examined. The living cells of both the green and chlorotic areas were frequently found filled with highly refractive bodies approximately 5 μ in diameter which were constantly in motion. Nevertheless, it is highly improbable that these bodies have any causal relation to the variegation, since they were found equally abundant in both the green and the chlorotic area. They were not observed in the fixed material.

2. *Ficus Parcellii* Veitch.—The variegation here is a mosaic-like mottling as shown in pl. 15, fig. 39. The different areas were very distinct, angular in appearance, and exhibited various distinct shades of green as well as pure white. Both living and fixed material were studied. Tissue taken from a nearly green leaf, such as that in pl. 15, fig. 45, was found to consist of the following layers of tissue,—upper epidermis, 2 layers of palisade cells, 4 layers of spongy mesophyll, and the lower epidermis, all of which contained chloroplasts. A study of the variegated leaves showed that the different colors of green had distinct

anatomical explanations, being due to a difference in distribution of the chloroplasts in the various layers. Transitional areas were always very sharp, one cell containing numerous chloroplasts, while the adjacent one might be completely devoid of them. It was found that there were 4 possible variants, as follows:

a. First palisade layer.
b. Second palisade layer.
c. First two rows of spongy mesophyll cells.
d. Lower two rows of spongy mesophyll cells.

Any one or any combination of these variants might be chlorophyll-free, while the remainder would be filled with chloroplasts. Some of the combinations which were observed are the following:

1. a, chlorophyll-free; b, c, and d filled with plastids.
2. b, chlorophyll-free; a, c, and d filled with plastids.
3. c, chlorophyll-free; a, b, and d filled with plastids.
4. b and d, chlorophyll-free; a and c filled with plastids.
5. d, chlorophyll-free; a, b, and c filled with plastids.
6. a, b, and d, chlorophyll-free; c filled with plastids.
7. a and b chlorophyll-free; c and d filled with plastids.

There was therefore no tissue differentiation between the green and the chlorotic areas, the difference in color being entirely due to an absence of plastids in one or more layers of tissue.

Cytological studies of both living and fixed cells revealed no inclusions comparable to those associated with the mosaic diseases.

3. Bougainvillea glabra Choisy.—The variegation in this species consists of a marginal chlorosis which may at times affect the entire leaf, as shown in the 3 leaves of pl. 16, fig. 48. The green portions of the variegated leaves, however, are not as dark a green as are the normal leaves, such as those shown in pl. 16, fig. 54. Microscopic studies showed that this had an easily interpreted anatomical explanation, which was similar to that given for the various shades of green found in *Ficus Parcellii*. The normal green leaf consists of an upper epidermis, one layer of palisade cells, 5 layers of mesophyll, and the lower epidermis, with chloroplasts distributed throughout the palisade as well as the spongy mesophyll cells. In the variegated leaves,

however, chloroplasts were never present in the palisade cells, being distributed only in the upper two layers of spongy mesophyll or through the entire mesophyll other than the palisade. The chlorotic areas lacked all chlorophyll. Here, again, the transition between the green and chlorotic areas was sharp, one cell containing normal plastids and the cell adjacent lacking them completely. Such transitions usually occurred near the veins. There was no difference in tissue differentiation between the two areas, the leaves being of uniform thickness.

Cytological studies on both living and fixed material gave no evidence of any intracellular inclusions which might be interpreted as the causal agency of the chlorosis.

4. Pittosporum Tobira Ait.—The variegated leaves are a duller green than the normal leaves and have a completely chlorotic margin of varying depth, as shown in pl. 15, fig. 40, which may be compared with the green leaf in pl. 15, fig. 41. Here the normal green leaves consist of a thick upper epidermis, 2 or 3 layers of palisade tissue, 8 or 9 layers of spongy mesophyll, and a lower epidermis. The chloroplasts are distributed throughout the palisade and the spongy mesophyll, being more numerous in the former. In the green areas of the variegated leaves the distribution of the plastids is different, there being 2 layers of chlorophyll-free cells immediately below the upper epidermis and another 2 rows of similar cells immediately above the lower epidermis. Accordingly, there may be said to be a chlorotic mantle surrounding the green tissue, thus accounting for the duller green color of the leaves. Funaoka ('24) has termed this type of chlorosis "periclinal variegation" and has described it in variegations of *Pelargonium zonale, Glechoma hederacea, Acer Negundo,* etc.

The variegated leaves show a white margin which is sharply set off from the dull green tissue. A microscopic study of this white area showed a notable decrease in thickness of the leaf, both the palisade layers and the spongy mesophyll being reduced. Particularly were the intracellular spaces reduced. In such areas the chlorophyll was completely lacking.

Cytological studies were made on both fixed and living material, and in neither the green nor the chlorotic areas could any unusual

intracellular inclusions be observed in the 100 sections examined.

5. Nerium Oleander Linn.—Here, as in *Pittosporum Tobira*, the variegation consists of a chlorotic margin of varying depths, as shown in pl. 15, fig. 35, which is to be compared with the totally green leaves as shown in pl. 15, fig. 36. Microscopic studies show that the normal green leaf and the green portions of the variegated leaves are similar, except that in the entirely green leaf there are more chloroplasts in the lower part of the spongy mesophyll than there are in the variegated leaves. The green tissue consists in the order mentioned of an upper epidermis, 2 rows of chlorophyll-free cells, 2 rows of palisade cells, 6 rows of spongy mesophyll, 2 rows of chlorophyll-free cells, and a lower epidermis. The chlorotic areas show the same tissue differentiation, but the chloroplasts are replaced by leucoplasts throughout the spongy mesophyll and the palisade cells. The boundaries between the green and the chlorotic areas are very sharp, one cell showing the normal chloroplasts and the one adjacent exhibiting only leucoplasts. These transitions are always coincident with the veins. Occasionally, definite areas are observed which are lighter green than the normal, and this was found to be due to the fact that such areas lack chloroplasts in the upper palisade layer or in the spongy mesophyll cells.

Living and fixed tissues were studied cytologically but no unusual inclusions were found in either the green or the chlorotic areas.

6. Coleus Blumei Benth.—It was considered that perhaps the variety "Mrs. Kirkpatrick" of *Coleus*, fig. 37, pl. 15, was closely related to the mosaic infections, since it showed the pronounced crinkling of the leaves which is so characteristic of the mosaic symptoms. Sections of the leaves were studied in both the fixed and the living condition, but no intracellular inclusions other than degenerated chloroplasts could be found. In the fixed material the chloroplasts occasionally assumed appearances comparable to the vacuolate bodies in the tobacco and petunia mosaics, but they did not give the proper staining reactions, becoming more blue than orange. In the living cells they were not at all comparable to the vacuolate bodies in tobacco and would never be confused with them. Clear, highly refractive

bodies were seen in the living cells and were in constant motion as though exhibiting Brownian movement. They were found, however, in both the green and the chlorotic areas and could not be interpreted as a causal factor in the variegation.

Anatomical studies showed a condition different from any which have been described in this paper. The transition between the green and the chlorotic areas was so gradual as to be scarcely perceptible. The green area possessed two rows of palisade cells which gradually became shorter and thicker until they could no longer be distinguished from the spongy mesophyll. Therefore, in the chlorotic areas there was no differentiation between the palisade and the spongy mesophyll.

7. Evonymus japonica Linn.—There were two different variegations of this species at the author's disposal, both of which were studied by Baur. The variety "*medio-picta*" is shown in fig. 49 of pl. 16, as contrasted with the normal green in fig. 50 and the other variegation, "*argenteo-variegata,*" in fig. 51. Anatomical studies revealed similar tissue differentiation in the 2 variegated varieties, so it will be necessary to include only a discussion of one of them, var. "*medio-picta.*"

In this variegation the difference in tissue differentiation is found to be quite similar to that described for the *Datura* plants infected with mosaic except that here there are 3 layers of palisade cells. Measurements were made and tabulated as in the study of the plants infected with mosaic.

TABLE X

COMPARATIVE MEASUREMENTS IN GREEN AND VARIEGATED EVONYMUS LEAVES

	Normal green	Variegated green	Variegated chlorotic
Thickness of leaf	215 μ	420 μ	320 μ
Thickness of upper epidermis	20 μ	25 μ	20 μ
Thickness of palisade	150 μ	175 μ	100 μ
Thickness of spongy mesophyll	125 μ	200 μ	185 μ
Thickness of lower epidermis	20 μ	20 μ	15 μ

These data show that the green leaves are much thinner than either the chlorotic or green portions of the variegated leaves, and that the difference lies chiefly in the palisade layer, which

is 3.5 times greater in the green areas of the variegated leaves than in the green leaves. In the chlorotic areas the palisade layers are greatly reduced and contain relatively few chloroplasts, the ones that are present showing signs of disintegration. The transitional area between two regions usually covers about 5 or 6 cells.

Cytological studies were made on both the living and the fixed material of both types of variegations and of the normal green plant. The chlorotic areas of both vars. "*medio-picta*" and "*argenteo-variegata*" showed vacuolate bodies comparable to those found in tobacco and petunia mosaics, except that they occurred only in mesophyll and never in epidermal cells. Although at least 100 sections of the normal green tissue were studied, no such inclusions were found in these at any time.

The bodies were very similar in appearance to those in tobacco infected with mosaic. They showed a strong affinity for Orange G, contained several large or numerous small vacuoles, could be found adjacent to, or independent of, the nucleus, and occasionally, in the fixed material, exhibited numerous dark blue-staining granules (see pl. 14, figs. 25–32). No indication of a limiting membrane could at any time be observed. In the living cells the bodies appeared very similar to those in the fixed material, except that granules were never observed in any of the vacuoles.

Therefore, although these vacuolate bodies were not found in the non-infectious variegations studied, they were observed in the variegated varieties of *Evonymus japonica*. This is one of the species in which Baur ('08) found infectious chloroses. This would, then, appear to be evidence in favor of the view that these cell inclusions are associated directly with the virus rather than with the chlorosis which results from the presence of the virus, since they have been found in connection with only the one type of chlorosis—the infectious type. However, the author favors the view that they are not the causal agency itself but rather the product of a reaction between the virus and the cytoplasm of the cells.

Summary

1. Epidermal and hair cells of leaves of tobacco plants infected with the mosaic disease were examined in living and fixed tissues. The following observations of Goldstein were confirmed: the vacuolate bodies were not associated with the nuclei but were carried through the cells in the protoplasmic streams, the plate-like crystals were independent of the nuclei, and they lost their typical structure when placed in chrom-acetic acid. Contrary to her observations, however, the writer failed to observe any autonomous movements in the vacuolate bodies, and only once could the appearance of a limiting membrane be identified.

2. The observations of Rawlins and Johnson with reference to the fact that the inclusions occurred more frequently in greenhouse plants than in those grown out of doors were confirmed.

3. Treatment of mosaic-infected tobacco plants with the longer or biological ultra-violet rays for 18 days caused a dwarfing of the plants and a masking of the symptoms. Cytological studies of the rayed plants showed that the inclusions were present as in the controls, and these observations led to the conclusions that the absence of bodies in plants grown out of doors is not associated with the ultra-violet rays which they receive from the sun's spectrum.

4. The filtered mosaic tobacco juice was inactivated by an exposure of 30 minutes to the abiotic rays, whereas, under similar treatment, a suspension of *B. prodigiosus* was killed in 30 seconds. This is considered as evidence against the theory that the causal agency is an organism.

5. Living epidermal and hair cells of *Petunia* presented excellent material in which to study the intracellular inclusions. In cells showing rapid streaming, the vacuolate bodies exhibited 2 different movements: a migration through the cell, due to their being carried in the streams; and a change in form. The latter movement was explained as resulting from a combination of the effect of the force exerted on the mobile body by the streaming protoplasm and the apparent changes in form due to its turning over in the streams.

6. Regarding the reactions to solvents, the vacuolate bodies

and the plate-like crystals were alike in their relatively high resistance to the action of formalin, HCl, and HNO_3, and in the solubility in KOH. They differed, however, in the fact that the crystals were soluble in 10–95 per cent alcohol and 1–10 per cent acetic acid, whereas the vacuolate bodies were soluble only in 95 per cent alcohol and were not touched by acetic acid.

7. Anatomical studies of mosaic-infected leaves of *Datura*, pokeweed, and *Aquilegia*, showed that the virus enhanced the development of some areas and inhibited it in others, neither area, therefore, being normal. The reduction of tissue in the chlorotic regions was localized particularly in the palisade layers, and the chlorosis was accompanied by a decrease in size and number of chloroplasts.

8. Cytological studies of *Datura* revealed in the chlorotic areas irregular, granular, and vacuolate bodies in association with the nuclei, comparable to those described in corn by Kunkel. They were not found in the dark green areas of the diseased leaves or in the healthy tissues.

9. Cytological studies of pokeweed revealed intracellular vacuolate and sometimes granular bodies only occasionally present in the chlorotic areas.

10. No inclusions, not also present in the healthy cells, were found in the chlorotic areas of the diseased *Aquilegia*.

11. Anatomical studies were made on seven different variegations, and the variations in the difference between the chlorotic and green areas in the various types of chloroses were observed.

12. No inclusions were found associated with the chlorotic areas in *Homalomena cordata* Schott, *Ficus Parcellii* Veitch., *Bougainvillea glabra* Choisy var. *variegata*, *Pittosporum Tobira*, Ait. var. "*variegatum*," *Nerium Oleander* Linn., and *Coleus Blumei* Benth. var. "Mrs. Kirkpatrick."

13. Cytological studies revealed the presence of vacuolate bodies in the mesophyll cells of the chlorotic areas of *Evonymus japonica* vars. "*argenteo-variegata*" and "*medio-picta.*" *Evonymus japonica* is one of the species in which Baur found infectious chloroses.

14. The vacuolate bodies, therefore, have been observed, as yet, only associated with the infectious chloroses.

15. These observations seem to justify the conclusion that the vacuolate and granular bodies discussed in this paper are associated directly with the causal agency rather than with the chlorosis which results from the presence of the virus in the plant. The author favors the view, however, that they do not represent the causal agency, but are rather the product of a reaction between it and the cytoplasm of the cells.

Acknowledgments

The writer wishes to express her sincere appreciation to Dr. B. M. Duggar for suggesting this problem and for his many kind and helpful criticisms throughout the work; to Dr. F. H. Ewerhardt, of Barnes Hospital, for the use of his mercury vapor lamps in the early part of the work; and to Dr. G. T. Moore for the privileges and facilities of the Missouri Botanical Garden.

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Explanation of Plate

PLATE 13

Fig. 1. *Petunia.* Living hair cell from leaf of healthy plant, showing only a nucleus and small plastids, and no inclusions. × 1000.

Fig. 2. *Petunia.* Living hair cell from the chlorotic area of a mosaic-infected leaf, showing, in addition to the nucleus and plastids, a typical vacuolate body near the plate-like crystal. × 1000.

Figs. 3–6. *Petunia.* Vacuolate bodies as seen in the living hair cells and epidermal cells. × 1000.

Fig. 7. *Petunia.* Rounded vacuolate body adjacent to the nucleus in an upper epidermal cell of the chlorotic area of a mosaic-infected leaf. The tissue was fixed in chrom-acetic acid, and stained with Haidenhain's haematoxylin and counterstained with Orange G. No formed structures or limiting membrane were present. × 2000.

EXPLANATION OF PLATE (*Continued*)

Fig. 8. *Petunia.* Irregular amoeboid-like vacuolate body in a mesophyll cell immediately above the lower epidermis. Fixed and stained as in fig. 7. × 2000.

Fig. 9. *Petunia.* Vacuolate body as seen in a hair cell which had been kept in 15 per cent alcohol for a 12-hour period. The resulting shrinkage left visible a distinct limiting membrane. × 1000.

Fig. 10. *Petunia.* Nucleus and adjacent body of a cell which had been held in 30 per cent alcohol for 12 hours, showing that this concentration of alcohol neither dissolved nor materially modified these vacuolate bodies. × 1000.

Fig. 11. *Petunia.* Vacuolate body in a hair cell which had been kept in 50 per cent alcohol for 12 hours, showing that there were no injurious effects. × 1000.

Fig. 12. *Petunia.* Vacuolate body in a hair cell which had been placed in 70 per cent alcohol for 12 hours, showing no solution nor disintegration even at this high concentration. × 1000.

Fig. 13. *Datura Stramonium.* Upper epidermal cell in chlorotic area of a mosaic-infected leaf, showing the indefinite granular body adjacent to the nucleus. Several dark-staining granules are present in it. The leaf was fixed in chrom-acetic acid and stained in Haidenhain's iron alum haematoxylin and counterstained with Orange G. × 2000.

Fig. 14. *Datura Stramonium.* Nucleus and adjacent body in an upper epidermal cell in the transitional area between the chlorotic and green area in fixed material of a mosaic leaf. The body shows some suggestion of a vacuolate structure, but no limiting membrane. × 2000.

Fig. 15. *Datura Stramonium.* Nucleus with adjacent body in an upper epidermal cell of the chlorotic area in a mosaic-infected leaf. The material was fixed and stained as in fig. 13. This is a rather typical appearance of the body, being filled with small vacuoles in which there were occasionally dark-staining granules. × 2000.

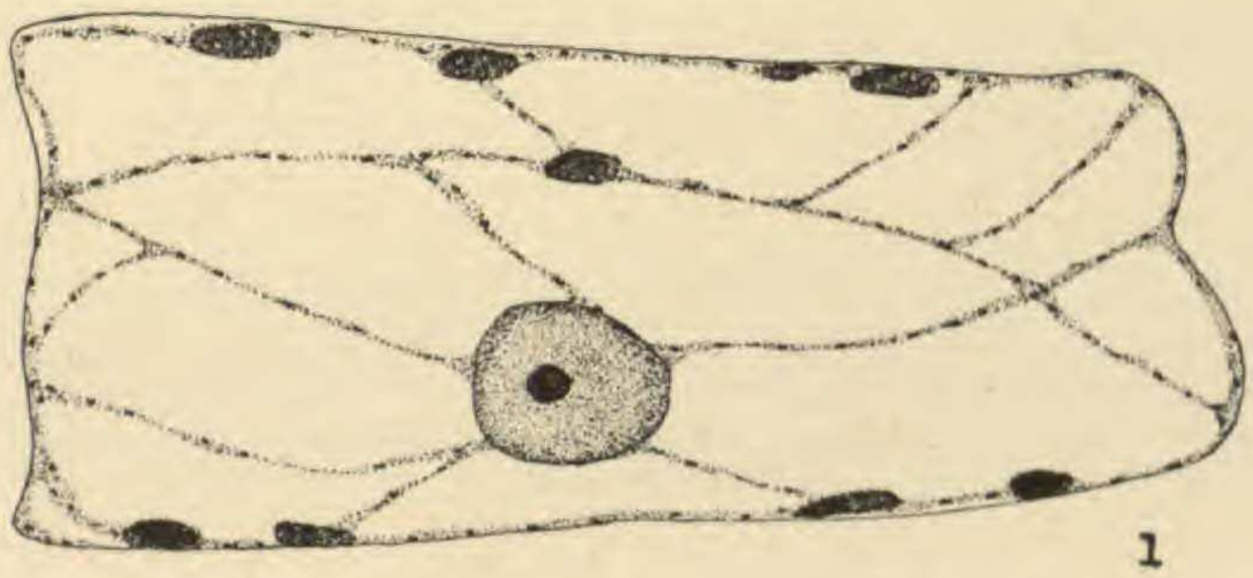
Fig. 16. *Datura Stramonium.* Apparently the young stage of a body. It was found in the upper epidermal cell in the chlorotic area of a mosaic-infected leaf which had been fixed and stained as in fig. 13. × 2000.

Fig. 17. *Datura Stramonium.* Unusual appearance of the body which completely surrounded the nucleus and was attached to the cytoplasm at the edge of the cell. Upper epidermal cell of chlorotic area. The body is less dense than usual and contains several dark-staining granules. × 2000.

Fig. 18. *Petunia.* Portion of hair cell from the chlorotic area of a mosaic-infected leaf, which had been placed in 4 per cent formaldehyde for a 12-hour period. The vacuolate body was still normal in appearance. The crystals had lost their sharp angular edges, and in the one on the right of the body disintegration and solution had set in. × 1000.

Fig. 19. *Petunia.* Portion of a hair cell from the chlorotic area of a mosaic-infected leaf which had been kept in 4 per cent formaldehyde for 1 hour. Both the vacuolate body and the plate-like crystals were normal in appearance. × 1000.

Fig. 20. *Datura Stramonium.* Healthy epidermal cell fixed in chrom-acetic acid and stained with Haidenhain's iron alum haematoxylin and Orange G. No inclusions were present. × 2000.



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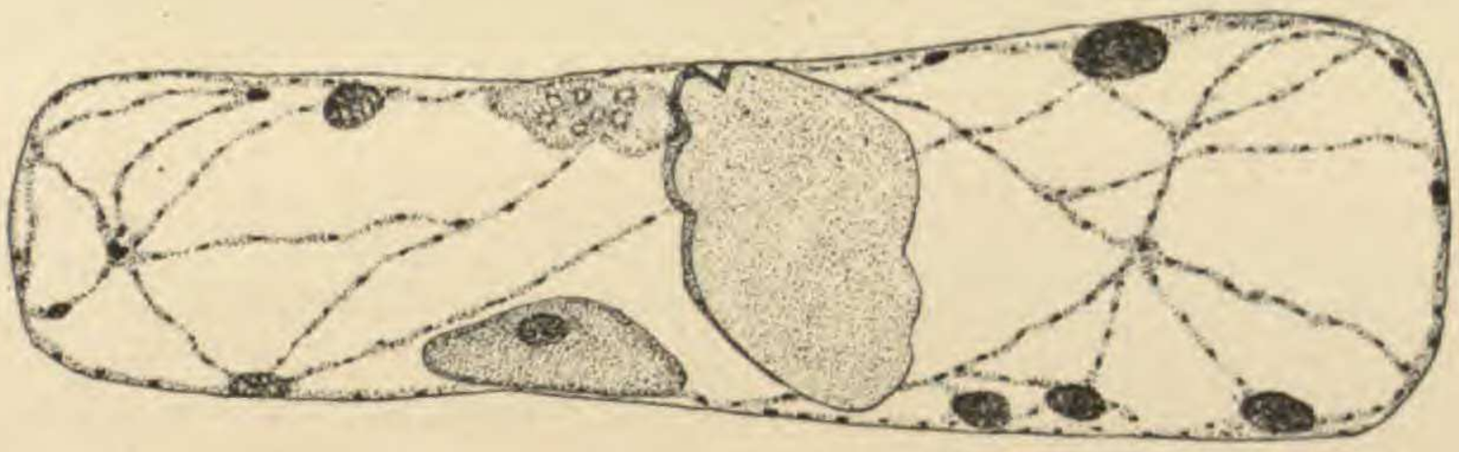

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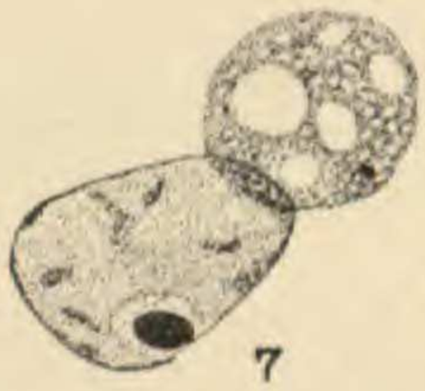

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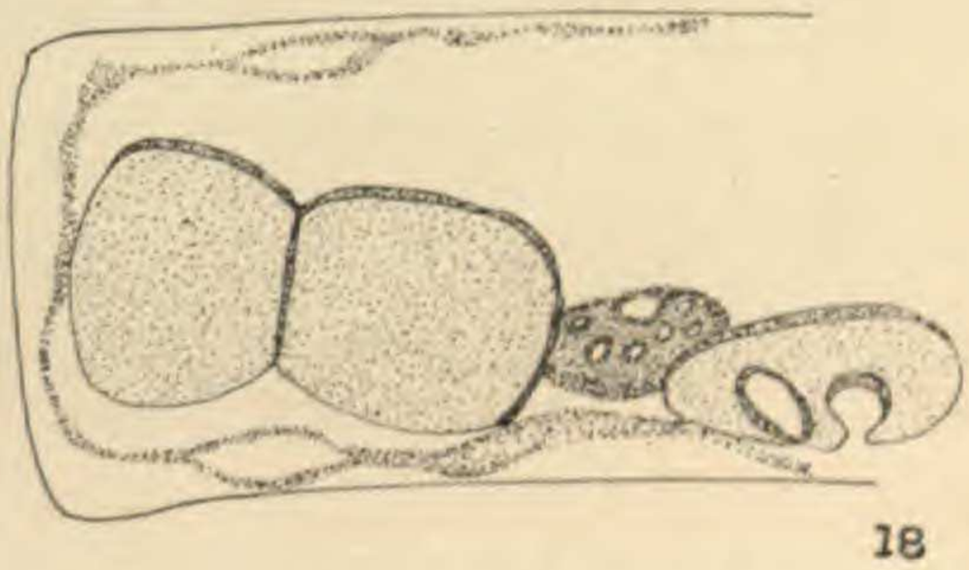

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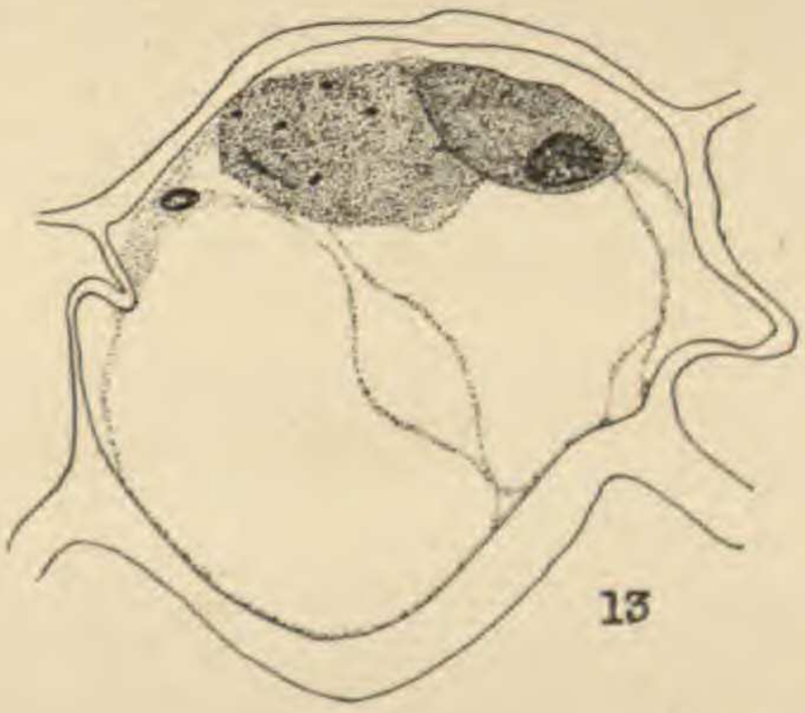

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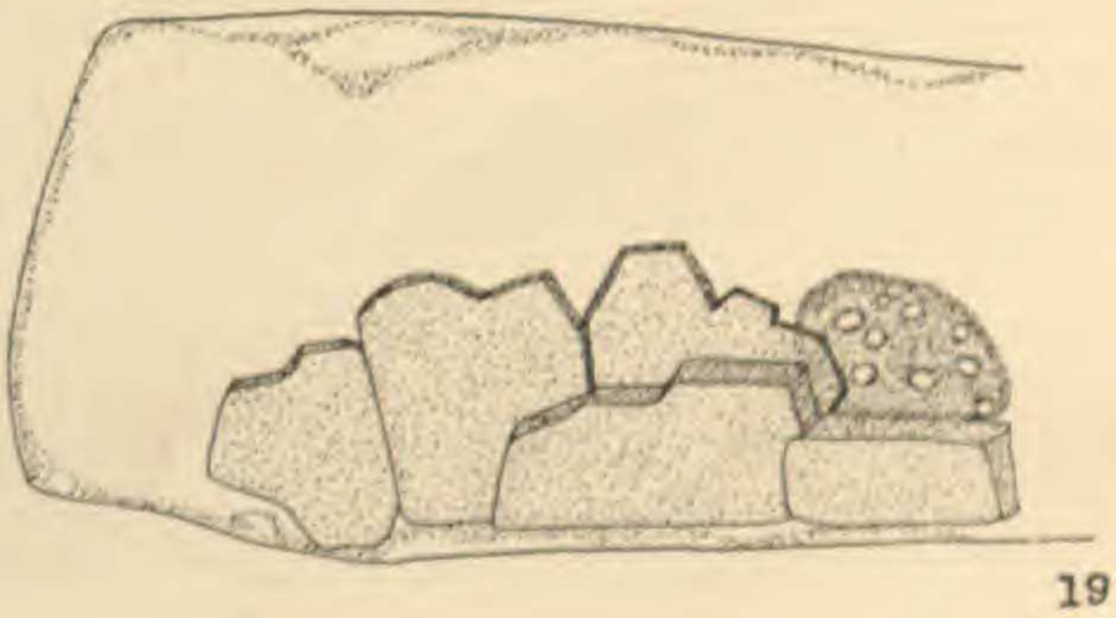

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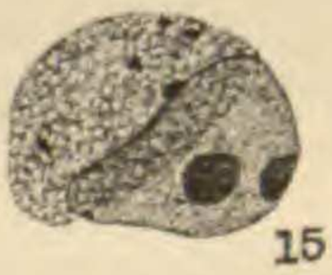

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SMITH—MOSAIC DISEASES AND LEAF VARIEGATIONS

Explanation of Plate

PLATE 14

Fig. 21. *Phytolacca decandra.* Upper epidermal cell of the chlorotic area of a mosaic-infected leaf, showing the very regular rounded body containing numerous small vacuoles, some of which surround very small dark-staining granules. The leaf was fixed in chrom-acetic acid and stained with Haidenhain's iron alum haematoxylin and Orange G. × 1500.

Fig 22. *Phytolacca decandra.* Cell immediately above the lower epidermis in the chlorotic area of a mosaic-infected leaf, fixed and stained as in fig. 21. This most unusual body was vacuolate, took a light gray-blue stain, was only slightly denser than the cytoplasm, and apparently was not associated with the nucleus. × 1500.

Fig. 23. *Phytolacca decandra.* Cell just above the lower epidermis in the chlorotic area of a mosaic-infected leaf, fixed and stained as in fig. 21. The cell showed the typical appearance of the bodies, the latter being a clear blue-gray and containing several large vacuoles. Several chloroplasts were also present in the cell. × 1500.

Fig. 24. *Phytolacca decandra.* Cell in second row above the lower epidermis in the chlorotic area of a mosaic-infected leaf. The very definitely walled structure in the center of the cell took a deep orange stain in the periphery which surrounded and gradually changed into the dark blue-staining centers. In addition, the cell contained 9 chloroplasts and a nucleus. × 1500.

Fig. 25. *Evonymus japonica.* var. "*medio-picta.*" Mesophyll cell in the chlorotic area of the variegated leaf. The body contained a large vacuole, numerous small ones, and several dark-staining granules. It apparently was not associated with the nucleus which was in the lower part of the cell among several plastids. × 1500.

Fig. 26. *Evonymus japonica* var. "*medio-picta.*" Nucleus and adjacent body which were found in a cell next to the one in fig. 25. The body contained a single large vacuole with several small ones. The material was fixed in chrom-acetic acid and stained with Haidenhain's iron alum haematoxylin and Orange G. × 1500.

Fig. 27. *Evonymus japonica* var. "*argenteo-variegata.*" Mesophyll cell in the chlorotic area of the variegated leaf, containing only the nucleus and a vacuolate body. There were no dark-staining granules in the body, and only 2 large vacuoles but many small ones. The material had been fixed and stained by the usual method. × 1500.

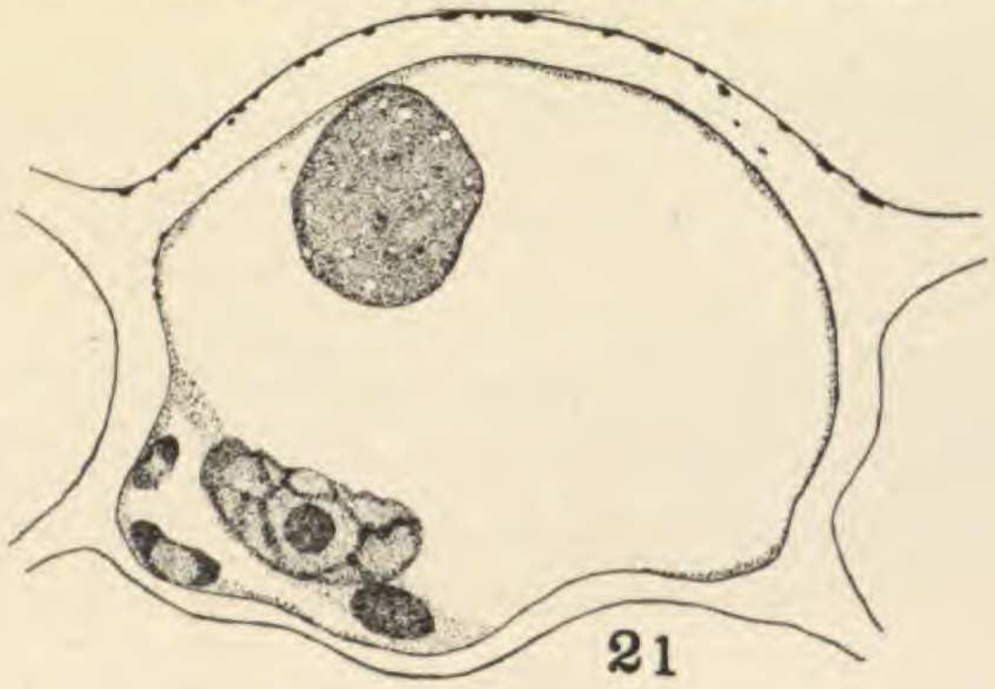
Fig. 28. *Evonymus japonica* var. "*argenteo-variegata.*" Vacuolate body in a mesophyll cell of the chlorotic area of a variegated leaf. It was surrounded by the nucleus and 2 disintegrating plastids. It contained two large vacuoles with dark-staining granules, and numerous small ones. The material was fixed and stained as in the previous figures. × 1500.

Fig. 29. *Evonymus japonica* var. "*medio-picta.*" Living mesophyll cell showing the nucleus with the vacuolate body partially superimposed. Two small chloroplasts were present in the cytoplasm. × 1500.

Figs. 30, 31, and 32. *Evonymus japonica* var. "*medio-picta.*" Vacuolate bodies as seen in the living mesophyll cells in the chlorotic areas of the variegated leaves. × 1500.

Fig. 33. *Petunia.* Portion of a hair cell from the chlorotic area of a mosaic-infected leaf which has been placed in 8 per cent formalin for 1 hour, showing the normal appearance of the vacuolate body but the beginning of disintegration of the crystals. × 750.

Fig. 34. *Petunia.* Hair cell from the chlorotic area of a mosaic-infected leaf which had been kept in 10 per cent HCl for 6 hours, showing that, although there had been very severe plasmolysis, neither the crystals nor the body were injured. × 750.



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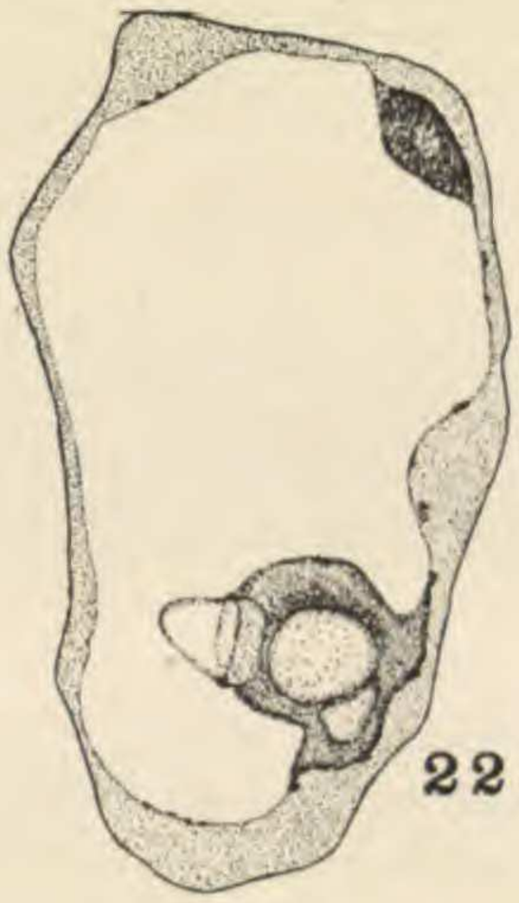

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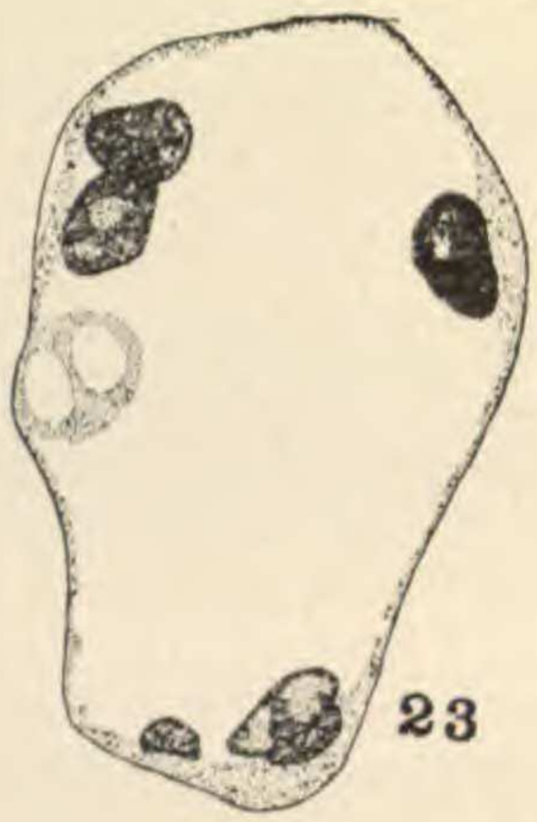

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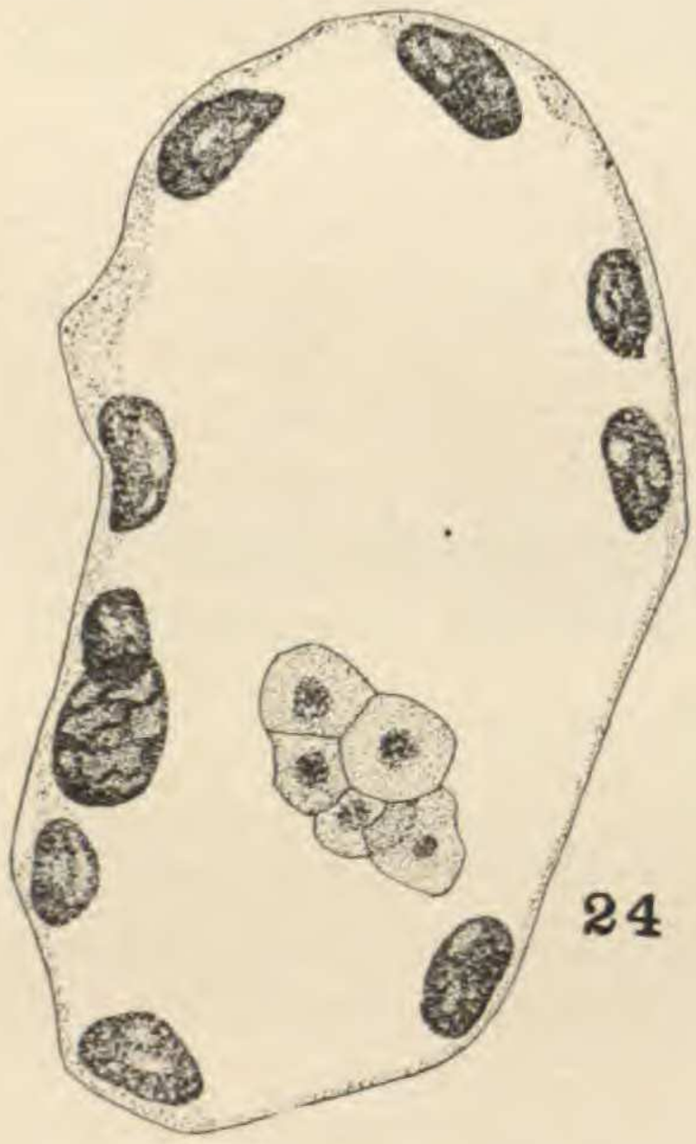

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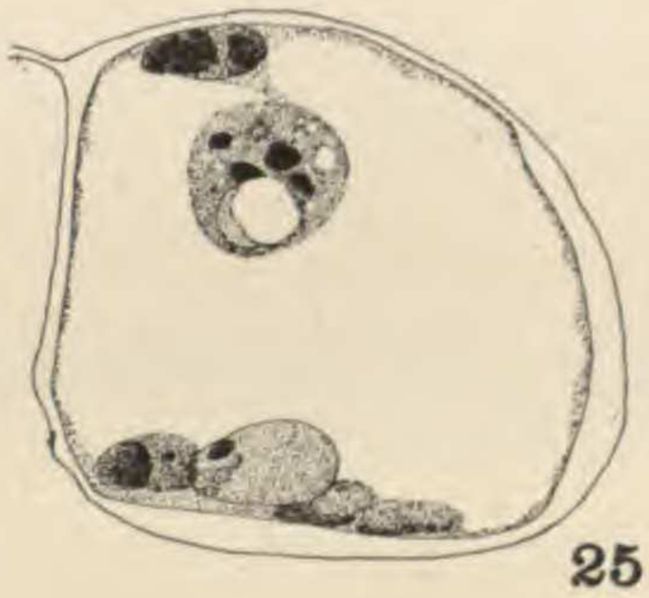

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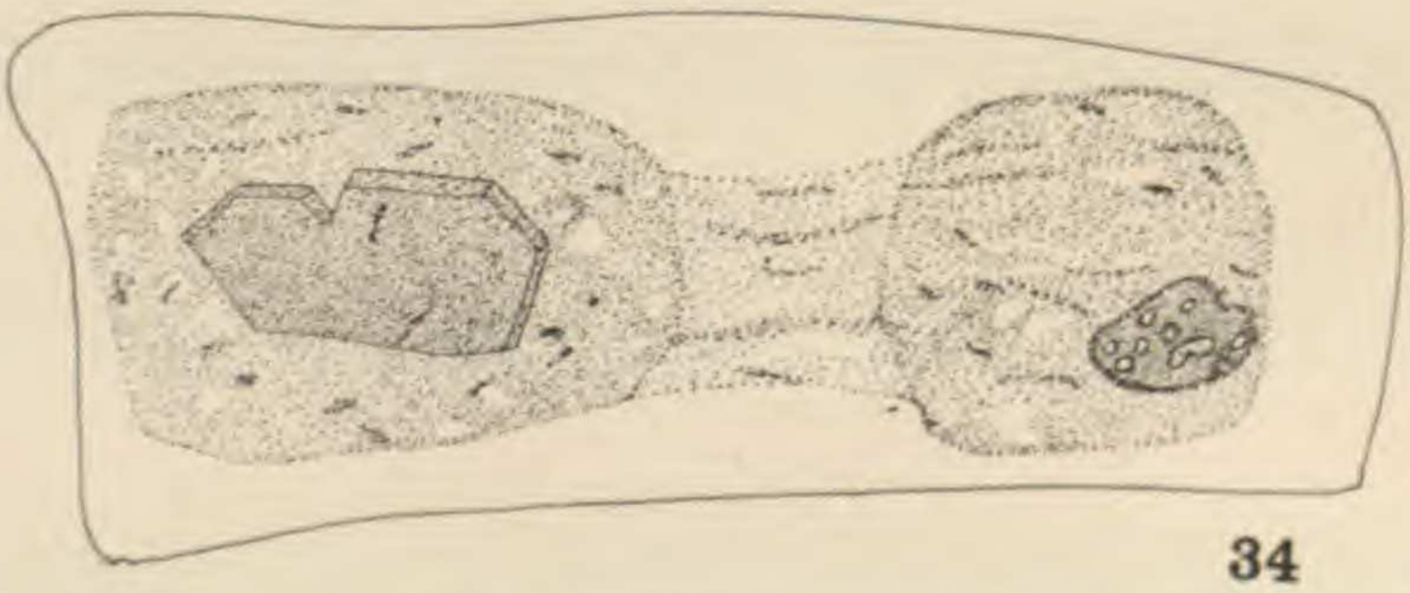

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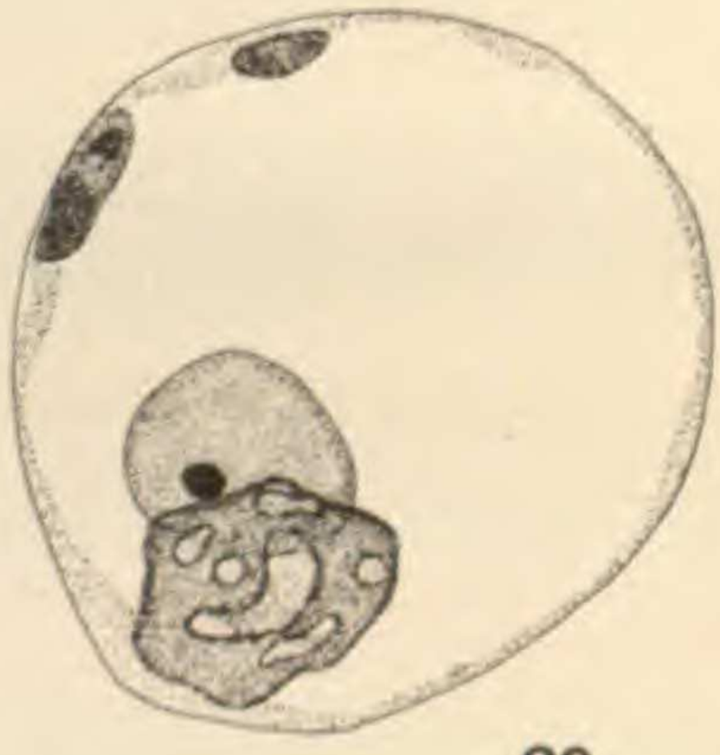

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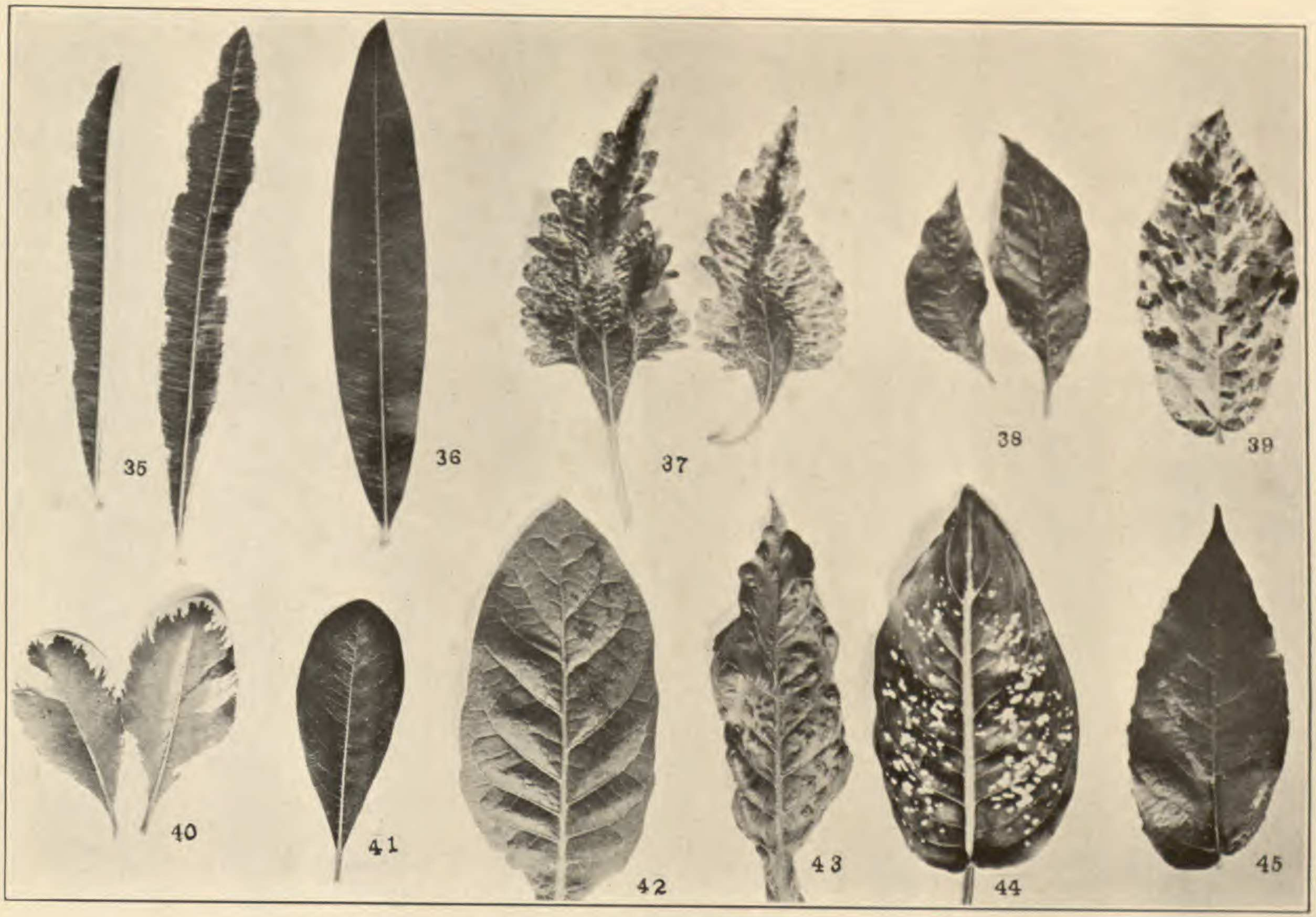
Explanation of Plate

PLATE 15

(Leaves one-half natural size)

Fig. 35. Leaves of the variegated variety of *Nerium Oleander* Linn.
Fig. 36. Normal green leaf of *Nerium Oleander* Linn.
Fig. 37. Leaves of *Coleus Blumei* Benth. var. "Mrs. Kirkpatrick."
Fig. 38. Mosaic-infected poke leaves.
Fig. 39. Variegated leaf of *Ficus Parcellii* Veitch.
Fig. 40. Leaves of *Pittosporum Tobira* Ait. var. *variegatum*.
Fig. 41. Normal green leaf of *Pittosporum Tobira* Ait.
Fig. 42. Healthy tobacco leaf.
Fig. 43. Mosaic-infected tobacco leaf.
Fig. 44. Leaf of *Homalomena cordata*.
Fig. 45. Nearly entirely green leaf of *Ficus Parcellii* Veitch.



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Explanation of Plate

PLATE 16

(Leaves two-thirds natural size)

Fig. 46. Leaves of mosaic-infected *Aquilegia caerulea* James.
Fig. 47 Leaves of healthy *Aquilegia.*
Fig. 48. Leaves of *Bougainvillea glabra* Choisy var. *variegata.*
Fig. 49. Leaves of *Evonymus japonica* var. "*medio-picta.*"
Fig. 50. Normal green leaves of *Evonymus japonica* Linn.
Fig. 51. Leaves of *Evonymus japonica* var. "*argenteo-variegata.*"
Fig. 52. Healthy *Petunia* leaves.
Fig. 53. Mosaic-infected *Petunia* leaves.
Fig. 54. Normal green leaves of *Bougainvillea glabra* Choisy.